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# Spacelab Life Sciences-1 Final Report

Bonnie P. Dalton, Gary Jahns, John Meylor, Nikki Hawes, Tom N. Fast, and Greg Zarow

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Nomenclatui	re	FEC	field engineering change
Symbols and Abbreviations		g	gravity
cfm	cubic feet per minute	GMP	guanosine monophosphate
fmol	femtomole	GPTU	general purpose transfer unit
g	gram	GPWS	General Purpose Work Station
Hct	hematocrit	GRF	growth hormone releasing factor
Hgb	hemoglobin	GSE	ground support equipment
mg	milligram	HEPA	high-efficiency particulate air
pmol	picomole	IL	interleukin
psi	pounds per square inch	JITS	joint integrated training simulation
Acronyms		JSC	Johnson Space Center
ACE	acetyl cholinesterase	KSC	Kennedy Space Center
AEM	Animal Enclosure Modules	L	launch
AN	arcuate nucleus	LCC	launch control center
ANF	atrial natriuretic factor	LMSC	Lockheed Missiles and Space Co., Inc. (now Lockheed Martin Missiles &
ANOVA	analysis of variance		Space)
ANP	atrial natriuretic peptide	LPO	lipid peroxidation
AOP	antioxidant protection	LSLE	life science laboratory equipment
ARC	Ames Research Center	MAb	monoclonal antibody
ATR	ambient temperature recorder	MAO	monaminoxidase
AVP	atrial vasopressin	ME	medial eminence
BFU-e	burst forming unit-erythroid	MAC	myosin heavy chains
BSP	biospecimen sharing program	MIT	mission integrated training
BTV	biotransport van	MITS	mission integrated training session
CDR	critical design review	MMO	mission management office
CFU-e	colony forming unit-erythroid	MPE	mission-provided equipment
CNP	C-type natriuretic peptide	MS	mission specialist
DFPT	delayed flight profile test	MSFC	Marshall Space Flight Center
DFRC	Dryden Flight Research Center	MVAK	module vertical access kit
ECS	environmental control system	N	number
EDL	extensor digitorum longus	NE	norepinephrine
Epo	erythropoietin	NIH	National Institutes of Health
ESA	European Space Agency	NSF	National Science Foundation
EUH	experiment unique hardware	OSSA	Office of Space Science and
EVT	experiment verification test		Applications
FD	flight day		

PCDT	particulate containment demonstration	SL-3	Spacelab-3
	test	SL-J	Spacelab Japan
PED	payload experiment developer	SLS-1	Spacelab Life Sciences-1
PI	principal investigator	SLS-2	Spacelab Life Sciences-2
POCC	payload operations control center	SLSPO	Space Life Sciences Payloads Office
PR	problem report		-
PRF	payload receiving facility	SMD	spacelab mission development
PS	payload specialist	SMMI	Small Mass Measuring Instrument
		SMIDEX	spacelab mid-deck experiment
PV	plasma volume	SPAF	single pass auxiliary fan
QA	quality assurance	SST	system sensitivity test
R + ML	recovery + mission length	STS	space transport system
R/IM	Refrigerator/Incubator Module	TEU	• •
RAHF	Research Animal Holding Facility		thermal electric unit
RAU	remote acquisition unit	TGF	transforming growth factor
	•	TNF	tumor necrosis factor
RBC	red blood cell	USSR	Union of Soviet Socialist Republics
RBCM	red blood cell mass	WBC	white blood cell

# Spacelab Life Sciences-1 Final Report

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Ames Research Center

## 1.0 Summary

This report provides a historical overview of the Spacelab Life Sciences-1 (SLS-1) mission along with the resultant biomaintenance data and investigators' findings. Only the nonhuman elements, developed by Ames Research Center (ARC) researchers, are addressed herein. The STS-40 flight of SLS-1, in June 1991, was the first spacelab flown after "return to orbit"; it was also the first spacelab mission specifically designated as a Life Sciences Spacelab. The experiments performed provided baseline data for both hardware and rodents used in succeeding missions.

Planning for SLS-1 started in 1978 with the Announcement of Opportunity (AO) from NASA Headquarters to the scientific community. Early hardware verification accomplished on Spacelab 3 (SL-3) with rats and monkeys pointed out some definite operational flaws. Although problems with particulate containment on SL-3 caused a major hardware impact on SLS-1, the mission delays allowed sufficient time for the development and verification of an upgraded, fully functional, animal loaded facility by 1991—the rodent Research Animal Holding Facility (RAHF). The delays also allowed an opportunity to compare two types of animal habitats, the RAHF and the Animal Enclosure Module (AEM), which are flown in the spacelab with individually caged animals and in the mid-deck with gang-caged animals, respectively. In addition, the SLS-1 flight verified the utility and functionality of the General Purpose Work Station (GPWS), the Small Mass Measuring Instrument (SMMI), and supporting hardware to transfer the live animals between the various pieces of equipment without the release of particulates. Charts are included to indicate postflight status of the hardware and actions implemented to prepare the hardware for succeeding missions. Although differing in some aspects, the spacelab hardware will provide models for the development of equipment for the Space Station era.

\*Lockheed Martin Missiles & Space, Moffett Field, Calif.

Data obtained from the hardware and the rats during the flight were compared to data obtained in a delayed flight profile test (DFPT) conducted immediately following the nine-day mission. Because of the lack of hardware availability, SLS-1 provided the only opportunity to obtain a RAHF ground control immediately postflight. Baseline biological data obtained from the flight and ground controls revealed that:

- Flight rats gained less body weight during the flight period than ground controls during the same period.
- Flight and ground rats gained weight at the same rate beginning two days postflight.
- No difference in body weights was noted between flight rats maintained in the RAHF and flight rats maintained in the AEM. Further discussion is provided on food and water consumption and organ weights.

Over 6,000 biosamples were distributed to the scientific community. Summaries of results obtained by the 10 primary investigators, along with those from investigators in the biospecimen sharing program (BSP), are included. This second group included investigators from various universities in Canada, Germany, Russia, and the United States.

### 2.0 Introduction

June 5, 1995, marked the fourth anniversary of the Spacelab Life Sciences-1 (SLS-1) flight. The results of the tests conducted on that flight could not be reported after the flight because completion of many of the experiments was dependent on activities of SLS-2. This report summarizes the scientific data from SLS-1 as an Ames Research Center (ARC) SLS-1 final report.

Abstracts from the experimenters are enclosed; the scientists summarized their results and listed publications and/or meeting proceedings in which the results were presented. The water, food-consumption, and weight-gain data retrieved from the flight and ground controls has been reviewed and analyzed, and varying aspects of these data are presented herein. The complete data sets are available from the ARC Life Sciences Data Archive.

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A summary of upgrades and/or refurbishment of the Research Animal Holding Facility (RAHF) hardware prior to its use on SLS-2 is included. The General Purpose Work Station (GPWS) was refurbished for immediate use on Spacelab Japan (SL-J), which flew in September 1992. Changes included replacement of the two-part sliding side window with a single-piece side window and installation of cabinetry electrical connections to accommodate microscope use and video downlink. The Refrigerator/Incubator Module (R/IM) door was also changed to support SL-J activities. All other hardware was transferred to subsequent flights "as is."

A six-month report was forwarded to Mission Management and Headquarters. The report, never formally published, is included herein as Appendix 1. With the exception of the rodent-body-weight data, no element of the SLS-1 90-day report (AR-01449) is included in this final report.

The SLS-1 ARC payload management extends a thankyou to all the principal investigators (PIs) for their cooperative efforts in providing information for this report.

Both the SLS-1 investigators and the ARC SLS-1 team acknowledge the excellent job of the SLS-1 crew: Bryan O'Connor, commander; Sid Gutierrez, pilot; Rhea Seddon, Jim Bagian, and Tamara Jernigan, mission specialists; Drew Gaffney and Millie Hughes-Fulford, payload specialists; and Bob Phillips, alternate payload specialist. Also acknowledged are the outstanding support efforts of all the personnel in the Space Life Sciences Payloads Office (SLSPO) and in other support organizations at ARC.

#### 3.0 Ames Research Center Hardware

#### 3.1 Background: 1978-1991

Hardware for the ARC experiments aboard SLS-1 started with concepts for animal holding facilities for rodents, squirrel monkeys, and rhesus monkeys and a GPWS as part of the Spacelab mission development test #3 (SMD-3) conducted at the Johnson Space Center (JSC) in 1977. The RAHF and GPWS were originally designed and built in the 1978 to 1981 time period for flight on Spacelab 4 (the term originally applied to SLS-1 and SLS-2), which was scheduled for a 1981 launch as the first dedicated Life Sciences mission. In the interim, RAHFs were flown as an "engineering proof of concept" aboard Spacelab 3 (SL-3) in April/May 1985.

Two versions of RAHF were built, one to house 24 rodents and one to house 4 unrestrained squirrel monkeys. The hardware was built at Lockheed Missiles and Space Co., Inc. (LMSC, now Lockheed Martin Missiles &

Space) and delivered to the Spacelab Life Sciences Payloads Office (SLSPO, then the Life Sciences Flight Experiments Project) in 1982. The GPWS was developed at the same time but was not delivered to the project until 1984 because of budget cuts and launch slips.

3.1.1 Research Animal Holding Facility (RAHF)- The RAHF was designed to provide for basic animal maintenance: air, food, water, waste management, lighting, humidity removal, and temperature control. Water was available to the animal in each cage compartment via a set of lixits mounted just above the cage top in the cage module. Food was dispensed via a feeder cassette mounted on the side of the cage; it required replacement by the crew every three days. Airflow directed urine and feces into a waste tray at the bottom of the cage. An Environmental Control System (ECS) mounted on the rear of the cage module controlled temperature and humidity. A water separator system removed excess humidity and transferred it to a condensate collector bag. When necessary, the crew changed the bag at a "quick disconnect" fitting. Lights were mounted just above the cage tops. Activity of each rodent was monitored via an infrared-beam activity monitor. A camera structure mounted over a four-cage segment on the rodent RAHF was activated during launch and reentry on SL-3. Figure 1 illustrates the SL-3 RAHF configurations (rodent and primate).

During the SL-3 flight, problems were encountered with the hardware; chief among these was particulate contamination and animal odor. Particulates observed by the crew and collected in fan filter screens in the Spacelab module included food-bar crumbs, fine charcoal bits, and fecal particles, which were released from the cage during feeder and waste-tray changeout. Persistent animal odor was also reported by the crew. At the direction of NASA's Associate Administrator for the Office of Space Science and Applications (OSSA) after the SL-3 flight, a committee was convened to review the design of the RAHF and recommend changes. Thirty-one discrepancies were noted with the design.

Extensive postflight testing of the RAHF hardware revealed several leak paths within the cage module, which prevented operation of the unit as a negative-pressure device. The outward direction of the air leaks accounted for the presence of odor in the cabin. The rodent cages were constructed without adequate sealing; e.g., the cage top was 1/4-in. grid, two holes in cage top for lixit access, waste trays not sealed at cage front, severely crumbing food bar, etc. Airflow was also highly erratic, turbulent within the cage, and nonexistent in some places.

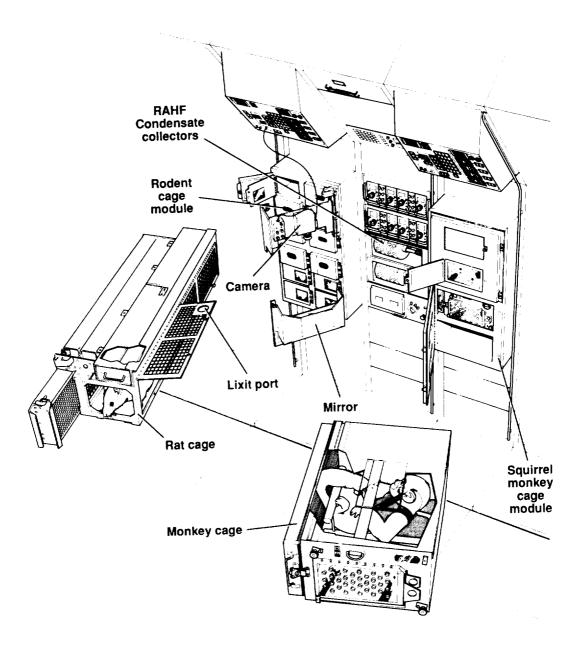


Figure 1. SL-3 RAHF configurations (rodent and primate).

As a result of the SL-3 problems, the RAHF was demanifested from the SLS-1 payload. The ARC experimenters proposed flying Animal Enclosure Modules (AEMs) instead so that the effect of microgravity on rats could be evaluated.

The RAHF was redesigned between 1985 and 1988 to prevent the recurrence of the particulate and odor

problems. New versions of the RAHF were delivered to the SLSPO in August 1988 and June 1989. Because of the launch delay to 1990, the RAHF was remanifested on SLS-1 in July 1987, after the critical design review (CDR) and unanimous acceptance of the new design by the crew and the oversight committee.

To assure requirements compliance with all elements in the redesign of the RAHF, a requirements document was developed and signed by the PIs, the Astronaut Office at JSC, the Mission Management Office for SLS-1, and the Life Sciences Division at NASA Headquarters. Hardware changes in the specification forwarded to LMSC included:

- Sealing the cage module to prevent odor escape and to insure inward airflow.
- Improving the ECS system to produce linear airflow through the cages.
- Redesigning the cage to include internal lixits, an improved waste tray, and a feeder with expanded food capacity.
- Assuring that all cage parts, including feeder, waste tray, and cage, are interchangeable (proven during SLS-1 flight integration).
- Sealing the cages to prevent escape of all particles >150 microns.

Modifications were implemented to alleviate various RAHF problems observed:

- Added single pass auxiliary fan (SPAF) to produce high inward airflow during cage servicing operations such as feeder or waste-tray replacement.
- Replaced all drinking-water-system parts with stainless steel. (The previous system had been susceptible to corrosion.)
- Added iodinator system to reduce drinking-water contamination.
- Implemented reliability upgrades as required in the water-separator fan and other critical components.
- Sealed cages to cage module to prevent escape of particles into the cabin. High-efficiency particulate air (HEPA) filters were installed to prevent escape of particles > 0.3 microns into the cabin.
- Addressed and corrected all problem reports (PRs) generated at the Kennedy Space Center (KSC) during the previous SL-3 integration activities.

Members of the Astronaut Office at JSC and the payload crew participated in the redesign activity. Special consideration was given to human-factors elements in the design, e.g., cage latches, SPAF configuration, waste-tray design, and the rodent-viewing window.

As a method of predetermining the RAHF airflow problems on SL-3 and altering them, an existing oil-pipelinedesign software program was modified to simulate the airflow in the RAHF. The program allowed analyses of

ineffective air paths in terms of leaks out of the module, and assisted in reconstruction of a system allowing sufficient air to the animals while insuring the capture of potential escaping particulates. During the development testing, airflow was greatly improved through the cages by placing a coarse mesh screen on the cage top, which served as a turning vane for air coming through the inlet plenum of the ECS. Testing with acetic acid smoke revealed that airflow was virtually linear over the length of the cage. The improved average 10-cfm airflow through the cages was in part due to the changed wastetray packing material. Use of Bondina, | charcoalimpregnated polyester foam, and Filtrete<sup>2</sup> facilitated airflow, eliminated loose charcoal, and maintained 150-micron particle containment, respectively. During SL-3, the use of layers of fiber glass batting and loose charcoal resulted in inconsistent pressure differentials across each cage and loss of charcoal particles into the cage module. The treatment of all filter materials with phosphoric acid was retained as a standard to prevent odor and eliminate microbial growth.

In addition to LMSC hardware changes, a low crumbing, 10-day-duration, wheat-based, microbial-resistant food bar was developed within the SLSPO along with a commercial means of production.

The RAHF was extensively tested at ARC. A 14-day biocompatibility test was conducted upon receipt of the unit. followed by a system sensitivity testing (SST), and an experiment verification test (EVT) 6 months later (March 1989). The crew participated in these tests, which included demonstration of the SPAF particulate capabilities, odor evaluation, and microbial-containment verification. All results were positive. Carbon-dioxide levels within the RAHF were also evaluated to insure conformance to less than 0.5 percent. The tests did reveal that animals would succumb to asphyxiation if there were loss of power and resultant loss of circulating air for periods greater than 45 minutes. This finding also verified that the unit was sealed tighter than the unit in SL-3, in which animals could be maintained for more than four hours in the absence of power and recirculating air. The second flight RAHF, which was delivered in 1990, underwent an extensive SST at Ames and was utilized during the delayed flight profile test (DFPT), a science control test at KSC. The second unit profile mimicked the first, which was integrated into the Spacelab. The SSTs characterized the performance of the RAHF, including responses to high and low fluid loop temperatures, responses to high and low ambient temperatures, and capabilities during

<sup>&</sup>lt;sup>1</sup>A porous filter made by Villdeon Filter, San Diego, Calif. <sup>2</sup>Made by 3M Filteration Products, St. Paul, Minn.

half thermal electric unit (TEU) performance. The data proved valuable as a diagnostic tool during pad and inflight operations. These data were, in fact, utilized as reference in requesting the lower coolant loop temperature prior to the insertion of animals during the third launch attempt. Figure 2 illustrates the features of the refurbished RAHF as flown on SLS-1. Only rodents (fig. 3) were accommodated in this tightly sealed unit, in which even the water lixits were internal to the cage.

Figure 4 illustrates a crew member checking the rats through the RAHF front windows during the SLS-1 flight.

**3.1.2 Flight diet**— The flight diet used in the feeding systems of both the AEM and the RAHF was developed to overcome the crumbing from the food bar used for SL-3. The SLS-1 formulation was a low-residue, defined food

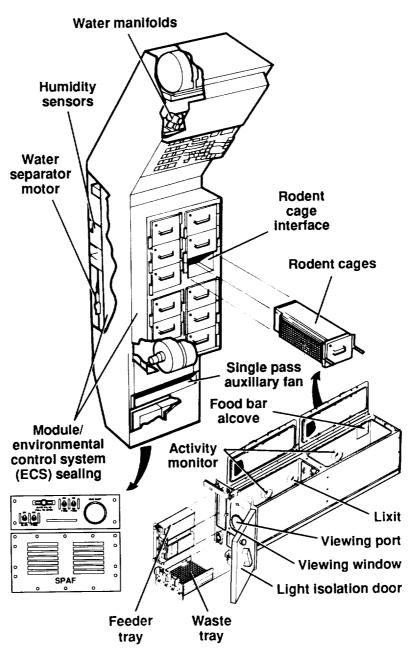


Figure 2. RAHF refurbished.

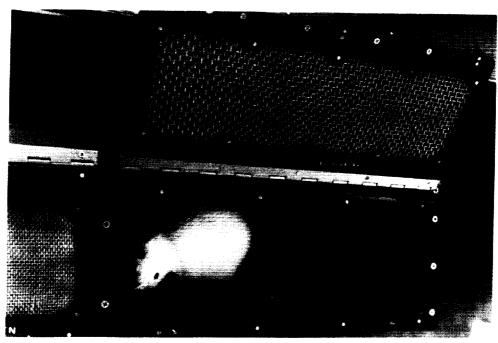


Figure 3. Rodent in RAHF cage.



Figure 4. Crew checking the rats in the RAHF.

bar<sup>3</sup> with an initial water content of about 26 percent. The manufacturing processes of milling/extrusion developed by the American Institute of Baking yielded a bar that reduced crumbing while maintaining the nutrient quality recommended by NIH and an energy yield of about 2.21 kilocalories per gram of prepared food bar. After the milling process the bars were treated with sorbate to prevent mold growth and radiation sterilized to prevent bacterial contamination. The processed food bars, packed in sealed bags, were held at 4°C to maintain nutritional value and palatability until used. Formulation for the diet was as follows (refs. 1–7):

NASA Experimental Rodent Diet (#TD 88179)	g/kg
Casein, high protein	100.0
DL-methionine	3.0
Wheat gluten	120.0
Wheat flour, durum 2nd clear	225.0
Corn starch	199.7349
Corn syrup	100.0
Sucrose	100.0
Corn oil	40.0
Cellulose (fiber)	50.0
Mineral mix, AIN-76 (170915)	35.0
Calcium carbonate	5.0
Vitamin Mix, AIN-76A (40077)	20.0
Choline bitartrate	2.0
Vitamin B <sub>12</sub> (0.1%) trituration)	0.23
Thiamin HCl	0.02
Folic acid	0.012
Menadione sodium bisulfite complex	0.0031

Wichadione sociatii bisainte complex	0.00	
Mineral Mix, AIN-76 (#170915) <sup>a</sup>	g/kg	
Calcium phosphate, dibasic (Ca <sub>2</sub> HPO <sub>4</sub> )	500.0	
Sodium chloride (NaCl)	74.0	
Potassium citrate, monohydrate	220.0	
Potassium sulfate (K2SO4)	52.0	
Magnesium oxide (M <sub>g</sub> O)	24.0	
Manganous carbonate	3.5	
Ferric citrate, USP (16.7% Fe)	6.0	
Zinc carbonate	1.6	
Cupric carbonate	0.3	
Potassium iodate (KIO <sub>4</sub> )	0.01	
Sodium selenite (Na <sub>2</sub> SeO <sub>3</sub> .5H <sub>2</sub> O)	0.01	
Chromium potassium sulfate	0.55	
(CrK(SO <sub>4</sub> ) <sub>2</sub> .12H <sub>2</sub> O)		
Sucrose, fine powder	118.03	

<sup>&</sup>lt;sup>a</sup>Designed to be used at 3.5 percent of diet.

Vitamin Mix AIN-76A (#40077) b	g/kg
Thiamin HCl	0.6
Riboflavin	0.6
Pyridoxine HCI	0.7
Niacin	3.0

<sup>&</sup>lt;sup>3</sup>Designed by scientists from Teklad, Inc., Madison, Wis.; Harlan Sprague Dawley, Inc., Madison, Wis.; and the National Institutes of Health (NIH), Bethesda, Md.

Calcium pantothenate	1.6
Folic acid	0.2
Biotin	0.02
Vitamin B <sub>12</sub> (0.1% trituration in mannitol)	1.0
Dry vitamin A palmitate (500,000 U/g)	0.8
Dry vitamin E acetate (500 U/g)	10.0
Vitamin D <sub>3</sub> , trituration (400,000 U/g)	0.25
Menadione sodium bisulfite complex	0.15
Sucrose, fine powder	981.08

<sup>b</sup>Designed for use at the 1-percent diet level (10g/kg). (This vitamin mix is designed without a choline source because choline bitartrate is listed as a separate item in the formula of the 88179 diet.)

The NASA flight food bar was established as an adequate nutrient source for rodents by its use in the Small Payload Program as well as in the Spacelab EVT. Animals fed this specialized form of the diet exhibited normal growth and apparent normal development. However, Danny Riley noted that the diet formulated for use on SLS-1 may have had only 50 percent of the vitamin B1 (thiamin) recommended by NIH and the National Academy of Sciences for laboratory rats. Since thiamine is necessary for preventing peripheral nerve degeneration, Riley was concerned that any changes found in the peripheral nerves of SLS-1 flight rats could be due to a diet artifact rather than space flight. Therefore, concentrations of thiamin were increased after SLS-1 and SLS-2 activities, and the enhanced food bars have been used in the SLS-2 formulation for all small payloads activities since 1992.

- **3.1.3 General Purpose Work Station** As a result of the anomalies of SL-3, the GPWS was re-evaluated, and the following changes were implemented during the period 1985–1988 to assure particle containment:
- Cabinet sealed to National Science Foundation-49 (NSF-49) Class II standards (contains particles
   <150 microns)</li>
- Side access window added to allow entry of small items such as rodent cage without opening the large front window and breaking containment
- Gauntlet ports added to front and side doors to prevent particulate escape during operation and to keep crew garments clean. Gauntlets were made of Tyvek,<sup>4</sup> a standard, medical, clean-room material. Gauntlets fit only to the wrist, thus allowing crew to use surgical gloves during delicate procedures. Spare gauntlets were installed in stowage, for use in the event of any tearing.

<sup>&</sup>lt;sup>4</sup>A fabric made by DuPont Fiber Division, Richmond, Va.

 Grille covers added inside cabinet to prevent particulates from entering HEPA filter system.

The GPWS was forwarded to the KSC in 1988 to allow sufficient time for modal testing in the flight-rack configuration. As a result of later, coupled-loads analyses, structural redesign was required, and the following changes were made:

- Two overhead stowage lockers eliminated and replaced by single closeout panel.
- Experiment power-distribution panel reconfigured to single panel spanning both sides of double rack.
- Bracing installed at interior corner posts.

Figure 5 illustrates the elements of the GPWS as configured for SLS-1.

3.1.4 General purpose transfer unit (GPTU)— An auxiliary piece of equipment, the GPTU, was developed as a result of particulate problems on SL-3. The GPTU was designed to accommodate transfer of rodent cages between the RAHF and the GPWS and thus eliminate any potential for release of particulates from the cage to the Spacelab environment. The GPTU resembles a windsock

attached to a lexan box frame (fig. 6), as seen during SLS-1. The frame attaches to the RAHF; a cage is pulled into the Tyvek windsock and closed off by a door in the lexan frame. The frame is then interfaced to the GPWS. Opening the GPWS side window opens the lexan frame window, and the cage is pulled into the GPWS. RAHF, GPWS, and GPTU interfaces were thoroughly evaluated during the EVT at ARC, prior to flight.

3.1.5 Animal Enclosure Modules— The two AEMs housed five rats each in the mid-deck location. AEMs had been flown on space transport systems (STS) 8, 11, 29, and 41 prior to SLS-1. AEMs are dependent on cabin air and circulation via internal fans for temperature control. The units remain closed during flight and, because of their configuration, there is no in-flight manipulation of specimens. Observations are made through a lexan cover. Food bars are glued to side walls; approximately 125 square inches of floor space are available.

Waste containment and absorption is accomplished through the use of a phosphoric-acid-impregnated, charcoal bed/filter pad. Temperature is monitored via an ambient temperature recorder (ATR), which is read postflight.

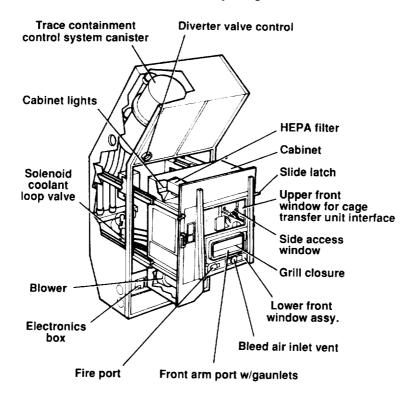


Figure 5. GPWS configured for SLS-1.

# ORIGINAL PAGE BLACK AND WHITE PHOTOGRAPH



Figure 6. General purpose transfer unit.

The ARC-constructed AEMs were modified from original units constructed by General Dynamics for the STS student program. The ARC units included a 1500-cc watering system and an automatic light timer.

Several changes were implemented in the SLS-1 AEM:

- Waste filter material changed to resemble that in the RAHF; resultant weight of AEM decreased about six pounds.
- Water box along with in-flight refill unit utilized allowed longer duration flight.
- ATR installed. Study of scrub 2 ATR playback resulted in preflight low-temperature conditioning of KSC biotransport van (58° C) and level IV carrier unit and request for continuing mid-deck 65° C air purge to launch -2 hours (L - 2).

**3.1.6 Small Mass Measuring Instrument (SMMI)**– The SMMI was a piece of JSC Life Science laboratory

equipment (LSLE) loaned to ARC. Three units were forwarded to ARC, one of which flew. ARC implemented a contract with Southwest Research Institute, San Antonio, Tex., the builders of the units, for refurbishment in 1989 because the units experienced continuous stability problems. Although the units were received from JSC as "flight certified" hardware, extensive additional testing was required by ARC to fulfill all elements of verification as defined in 1986. The SMMI was flown in SLS-1 to verify its calibration-maintenance capabilities before its use as experiment support in SLS-2. The units continued to perform well throughout SLS-2.

3.1.7 Refrigerator/Incubator Module— The R/IM was procured as an addition to an existing Marshall Space Flight Center (MSFC) contract. MSFC units had been flown earlier in numerous missions since STS-26 supporting microgravity materials experiments. As MSFC had done, ARC replaced various electrical components and incorporated a digital temperature readout. For SLS-1, the

mid-deck configured unit was flown in Spacelab in the Spacelab mid-deck experiment (SMIDEX) rack configuration. The unit was maintained at 28° C and supported the jellyfish flasks and bags.

3.1.8 Miscellaneous stowage - Various stowage hardware onboard was modified, commercially supplied items, e.g., air sampler and video camera. The air sampler was a copy of units utilized previously for microbiological sampling aboard the STS. The agar strips, normally used for microbiological sampling, were removed. A fine mesh screen, entrapping particles >150 microns, was attached over the minicentrifugal head. The screens were covered with a solid lid at the conclusion of each sampling, and the unit was screwed off the sampler and retained in stowage for observation at the end of the mission. The video camera was outfitted with a special adapter plate, which allowed handling of the jellyfish flasks in a steady, mounted position. The jellyfish bagging system was a combination of syringes mounted within sealed bags. Development of equipment supporting the jellyfish experiment (R/IM, video brackets, bagging system) was not started until 1986, when the experiment was manifest aboard SLS-1.

Another type of stowage, which served as accessories to the AEMs and the R/IM, were the ATRs. These units are the size of the European Space Agency (ESA) type 1 containers, have a wide temperature range, and are battery maintained for several months. The units can also be configured with external probes, if required.

#### 3.2 Results

3.2.1 Research Animal Holding Facility— The RAHF was flown with 19 animals of approximately 250 grams each. One cage compartment (6B) was flown empty because of lixit failure on the launch pad. Two of the other cage slots, 2A/B and 9A/B, contained equipment for the particulate containment demonstration test (PCDT). With the exception of the pressure transducer anomaly (detailed under Section 3.3, Anomalies), the RAHF performed as planned. Figure 7 illustrates the "on pad" T – 0 data (launch control center (LCC) prelaunch data), which included monitoring of quadrant 1 temperature, humidity 1, TEU coolant inlet temperature, and coolant flow status. The following facts were observed:

- High quadrant temperature (27°C) noted on launch attempt two was attributed to sustained mission-provided equipment (MPE) fluid loop temperatures of 21°C. The MPE loop was reduced to 12–14°C, and nominal temperature data were received and maintained to L – 6 hours.
- Leak alarms noted after launch attempt two. Module vertical access kit (MVAK) technicians were able to

reset 4A and 4B. Cage 6B could not be cleared; no animal was placed in that cage slot during launch attempt three (only 19 animals flown in RAHF). The RAHF was maintained on "ON" condition between launch attempts two and three.

(Note: Rodents were lowered into the RAHF at approximately L-29 hours on both launch attempts two and three).

Figure 8 typifies the RF1 and RF2 (designates in-flight data) responses observed throughout the flight and processed through the ARC ground-data compilation. Temperature and humidity matched ground tests, but quadrant four data were slightly lower than expected. Raising the set point to 25°C (from 24°C) brought all temperatures to nominal limits. The MPE fluid loop was approximately 12°C.

The water-tank pressure transducer failed on flight day (FD) 3. Three activity monitors failed in flight; the data were redundant with water counts. Two computer crashes of approximately 5 hours each interfered with data retrieval. Because of the uncertainty of water consumption versus water availability, the crew added Gel Paks to the cages on FD 8. The following data, retrieved at the end of the mission, very closely mimicked the data obtained with the second RAHF used during the delayed flight profile test (DFPT) conducted at Hangar L at the KSC facility 30 days postlanding:

- Total condensate collected during the flight:
   ~3.5 liters
- Microbial analysis of condensate: Pseudomonas paucimobilis
- Microbial analysis of water tank: No colony forming units
- Total water retrieved from water tank (includes MVAK operations and postflight micro sample volumes): 3.8 liters.
- **3.2.2 General Purpose Work Station**—The GPWS was used in flight for performance of the PCDT when both particulates and fluids were released on two different days by two different crew members. In addition, the GPWS was also utilized for:
- Observation of in-flight release by crew member of a rat from cage within the GPWS cabinet (FD 7)
- Addition of Gel Paks to each rodent cage compartment (FD 8)
- Fixation of jellyfish specimens within their bag system (FD 9)

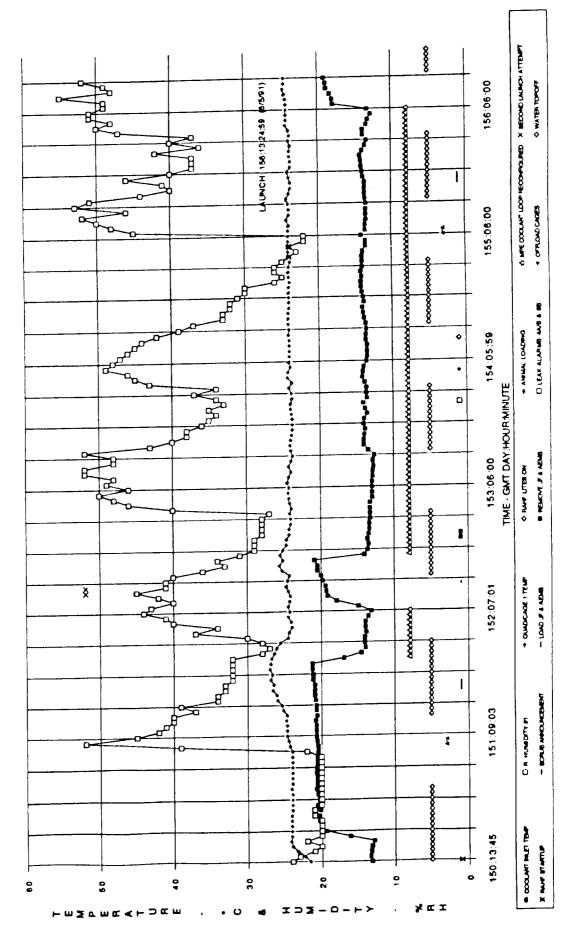


Figure 7. LCC prelaunch data.

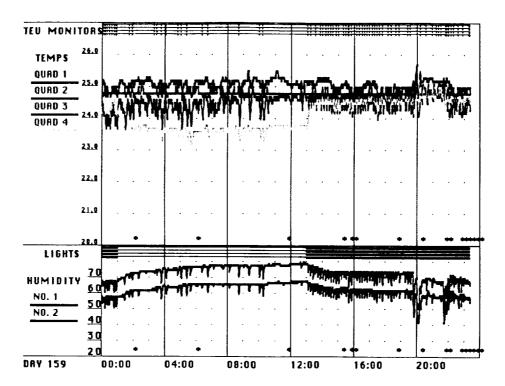


Figure 8. RF1 and RF2 responses.

All activities with the GPWS were nominal with the exception of several crew observations indicated in Section 3.3, Anomalies.

After the initial particulate dispersion, a crew member reported particulates settling via the airflow within 20–30 seconds. Initial dispersions resulted in some adherence to interior surfaces, which was thought to be due to static attraction. This condition was not observed during the second dispersion; particulates were readily flipped from surfaces with a plastic bag. A long-handled brush was incorporated in subsequent flight stowages to aid in cleanup.

Postflight microscopic examination of the centrifugal sampler screens collected during both GPWS and RAHF PCDT activities revealed particulate accumulation under only one condition and on only one screen at a size level of <50 microns and not exceeding 20 particles/inch. That condition was during the first release and cleanup within the GPWS when the crew failed to adequately clean the interior backside of the GPWS front window and material was entrapped when the window was raised. With appropriate cleaning operation, the condition was not repeated during the second particulate release.

The crew kit (ping-pong ball), implemented at a crew member's request, proved extremely beneficial in demonstrating airflow patterns and the appropriate window height for retrieval of items without contamination to the Spacelab atmosphere.

The PCDT, involving both the RAHF and GPWS, was so successful that the Administrator approved transfer of live rats in their cages from the RAHF to the GPWS for handling within the GPWS (fig. 9). This opportunity provided useful insights on animal behavior outside their smaller, closed environment (RAHF cage). It also demonstrated debris when the cage was opened in the GPWS since there was no airflow through the cage outside the RAHF. Procedures were implemented to minimize this release within the GPWS and thus not contaminate any processed samples within the GPWS during SLS-2 experiment activities.

Although the jellyfish experiment bagging system was triple-contained, the STS Safety Committee requested that the GPWS be used for the fixation activities (fig. 10) "...because it was available." The requirement to start up

# ORIGINAL PAGE GLACK AND WHITE PHOTOGRAP ...



Figure 9. Rodent within GPWS.



Figure 10. Jellyfish activities in the GPWS.

the GPWS and transfer all activities to the cabinet unnecessarily impacted available crew time.

**3.2.3 Refrigerator/Incubator Module**— The R/IM maintained its preset 28°C temperature throughout the flight and adequately maintained the jellyfish kits, which were placed within the R/IM.

**3.2.4** Animal Enclosure Modules— The two units performed nominally. Though lexan windows were extremely soiled by FD 3 (also observed in previous and subsequent flights) and alarming amounts of debris were viewed floating with animals, the AEM animals appeared well groomed on return and exhibited food consumption, water consumption, and weight gain comparable to that of RAHF animals. The data are discussed in more detail in Section 5.0, Science Results.

Data obtained from the ATRs postflight have been compared to results from the small payloads flight of the AEM over several years. There were indications that middeck temperatures and location of the AEM within the middeck greatly affect the AEM temperature profile. Results of this study are not included herein.

The in-flight refill capability allowed use of the AEMs for the extended flight. Normal capacity is limited to a maximum of 6 to 7 days with the 1,500-cc bladder. Units are currently filled directly from the crew's potable-water source.

**3.2.5 Small Mass Measuring Instrument**– Performance of the SMMI exceeded expectations. The data shown in the following table were recovered from operations performed on FD 4 and FD 6 with predetermined weights:

Actual mass	175.21 g	250.21 g	100.21 ± 175.21 g
Measured mass trial #			
1	175.0	250.2	275.3
2	175.2	250.4	275.1
3	175.1	250.1	275.4
4	175.2	250.2	275.4
5	175.2	250.2	275.4
6	175.3	250.2	275.4
7	175.3	250.2	275.3
8	175.3	250.1	275.3
Average	175.2	250.2	275.3

#### 3.3 Anomalies

Four anomalies were noted against the ARC hardware during the SLS-1 mission and reviewed by the Head-quarters (code UL)-appointed Robbins Committee. The first three anomalies were closed out by the committee; the fourth remains open for further resolution by ARC:

- Failed lixit, cage 6B
- RAHF leak alarms 4A, 4B, and 10B in flight
- AEM swagelock fitting loose
- RAHF water-pressure-transducer failure

The history of these anomalies follows:

3.3.1 Failed lixit, cage 6B— During the third launch-attempt MVAK operations, leak alarms were noted on cage slots 4A, 4B, and 6B. The MVAK technician was able to successfully reset 4A and 4B; 6B did not respond, even after 180 cc of water was manually drained through the lixit. No animal was placed in the 6B cage slot because of the inoperative lixit.

Postflight testing revealed that the problem was due to air in the lines. Removal of the air resulted in nominal functioning and calibration of the lixit. Corrective action required burping of the water manifold during the integration process to eliminate air. The procedure had not been performed because of integration-processing schedule conflicts.

Subsequent missions were not affected because appropriate planning, (e.g., procedure was included in Ground Integration Requirements Document) and correct integration burping procedures were scheduled and implemented. For those leak alarms occurring as a result of rapid water consumption by the rat or bumps against the lixit, ARC is designing a monitoring system for use during preflight; it will allow for tracking of water counts and a remote master reset of leak alarms from the LCC console.

## 3.3.2 RAHF leak alarms 4A, 4B, and 10B in flight-

Leak alarms in cage slots 4A and 4B were discovered on FD 1 during Spacelab activation. A leak alarm also occurred in cage slot 10B on FD 2. The RAHF water system was designed to shut the lixit off if greater than eight counts were received in an eight-second period. During the ARC biocompatibility and verification tests, three leak alarms were experienced during each test. Although the system performed nominally, changing it would have been counteractive to required safety constraints, so no corrective action was implemented.

3.3.3 AEM swagelock fitting loose—On FD 5 it was necessary to refill the AEMs. AEM 1 was filled nominally; a water leak appeared around the swage fitting on the refill lines when AEM 2 filling was attempted. The maximum volume of water released, as reported by the crew, was 0.25 to 0.50 cc. The crew was able to hand-tighten the fitting completely and eliminate any leaks. The second filling on FD 8 was without incident.

Evidently inspection of lines and fittings during the preflight preparations was inadequate. Appropriate inspection points in assembly procedures eliminated the problem for future flights. The corrective action involved evaluating preflight assembly and processing procedures and inspection lines to insure proper hardware configuration.

# 3.3.4 RAHF water-pressure-transducer failure-

Historically the RAHF water-pressure transducer has functioned with high reliability. This pressure transducer operated nominally during all functional testing both at ARC and KSC, and through all testing and refill operations performed during levels IV through I (on the pad). On FD 3, the RAHF-transmitted readings of water-tank pressure went from 36.8 psi to 55 psi . Evaluation of the "raw voltage" showed a constant reading of 102 psi, which is full scale.

As part of the failure analyses, the RAHF and other systems were tested postflight outside the Spacelab but in the flight rack configuration. The following tests were made:

- RAHF powered with ground-support equipment (GSE): The transducer read 22–18 psi, which matched the 3.4-liter volume left in the tank.
- Flight remote acquisition unit (RAU) tested with ground unit tester, which applied voltage through the unit and verified channel response; all elements performed nominally.
- RAHF/RAU interface tested by applying GSE power to determine if translational voltage from transducer to RAU (or reverse) could have resulted in failed readings and the 102-psi voltage indication; both the RAHF transducer and the RAU performed nominally.

In conclusion, this anomaly is unexplained. The RAHF was returned to ARC from KSC the week of November 8, 1991. Testing continued to resolve the issue prior to SLS-2 use. ARC continued to use high-reliability parts and installed a manual gauge for direct readout, in the event that a similar anomaly occurred during SLS-2. All pressure systems performed nominally during SLS-2, including repressurization during water refill of the RAHF water tanks.

**3.3.5 Other issues** – Other issues referenced during crew debriefings and various reports are noted as follows:

PCDT particles stuck in GPWS grilles

Care should be observed not to push large items through grilles. Items larger than the grille width were not intended to be pushed through the grilles. SLS-2 procedures reflected these cautions.

PCDT particles stick to GPWS door

A long-handled cleaning brush was installed in SLS-2 stowage to facilitate cleaning in corners, in crevices, and on inside of door face.

GPWS rails bind and interfere with GPTU/GPWS mating

The rails on the GPWS side window used in SLS-2 were reworked. SL-J used a plain window.

Dirty velcro in GPWS

Although the use of a double-backed velcro that could be easily replaced in flight was studied, the velcro was not replaced.

· Gauntlets limit visibility

The crew did not use the garters provided; SL-J uses a rubber band to curtail ballooning effect of gauntlets. ARC investigated elastic shirring down gauntlet side to minimize ballooning for SLS-2.

RAHF adapter rails loose

Detents were tightened prior to SLS-2 with positive latch.

Slide valve on RAHF SPAF

The RAHF office investigated a variable flow capability on the SPAF to reduce the potential for feces from cage front waste compartment to drift to back compartment during SPAF activation.

· Tight foam around AEM refill unit

This problem was reported in previous flights. More project interaction with the Boeing flight equipment processing contractor (FEPAC) was recommended along with "fit checks" prior to shipment of foam inserts to KSC.

Heightened AEM preflight temperatures

ARC implemented procedures to circumvent elevated temperatures in the AEM, including cooling the biotransport van (BTV), purging the mid-deck with 65°F air to as late as possible prior to launch, and using only half of the lights. Prior to the use of the ATRs in the AEMs, these preflight elevated temperatures were not "apparent."

GPWS phase imbalance

The GPWS was retested with a quality assurance (QA) witness during SL-J integration. There was no phase imbalance.

· GPWS low flow light

The "LO FLO" light was activated on the last flight day during the jellyfish fixation activities in the GPWS. Two possibilities existed to explain this anomaly:

- inefficient opening of grille closures;
- suspended particulates in the system, which blocked the system.

The GPWS was activated on return to 1 g, the grille closures, though difficult to open, were operated in the "OPEN" position, and the unit performed nominally.

All PRs and field engineering changes (FECs) generated at KSC were reviewed prior to refurbishment of any hardware utilized in succeeding missions.

3.3.6 Lung-tissue analysis- The flight crew of SLS-1 had commented that particulates were floating in the AEMs in zero gravity and had indicated a concern that these debris were being aspirated into the rodents' respiratory tree. To determine if this was a cause for concern, pathological examinations of the respiratory trees of 5 flight and 5 control rats were made by a veterinary diagnostic laboratory. The analyses for type of debris, size of particles, profile of location, and associated anomalies were done blind. Very rare intra-alveolar fragments of debris found in 6 of the 10 animals were limited to 1 to 3 fragments in the sections examined. Intraalveolar hair fragments were found only in 2 control specimens. Also found was a tiny, sharp, crystalline shard that was unidentified because of the small size and limited quantity present. Congestive changes consistent with decapitation were noted, as were peribronchiolar accumulations of small numbers of lymphocytes and rare plasma cells. In summary, no differences were noted between the flight group and the control group.

#### 4.0 Crew Training

Training began in September 1987 and continued until the launch of STS-40 on June 5, 1991. As of August 1988, the mission management office (MMO) was distributing schedules showing a June 1990 launch date. Consequently, training schedules reflected that July 1988 was

L-23 months and ARC was preparing to coordinate training for the SLS-1 payload crew. The generic training template used by ARC to schedule training was difficult to follow because of several launch slips and hardware and crew unavailability.

It should be noted that the payload crew had already begun training on SL-4 experiments in the fall of 1983. When training resumed in the fall of 1987, the original SL-4 payload had been reduced to hardware verification of the RAHF, RAHF adapter, GPWS, GPTU, and SMMI. (RAHF, GPWS, and GPTU verification was to be accomplished through the PCDT). In addition, crew in-flight activities concerned with RAHF/AEM rodent health observations, AEM water refill, jellyfish inducement and fixation, and jellyfish filming were scheduled.

# 4.1 Ames Research Center Training

The ARC mission-dependent training is divided into timed phases: orientation, task, phase, project integrated, mission integrated, and proficiency. Every component of each experiment and associated hardware is subject to the same basic training template. This approach provides an ideal working model as each successive training session builds on knowledge gained from the previous session until proficiency on integrated payload procedures is achieved.

The obstacles that greatly affected the training program were hardware availability, changing in-flight requirements, and launch slips. Every launch adjustment caused fluctuations in mission-specialist (MS) support and required additional resources to bring all individuals to a similar level of proficiency. In addition, hardware development and verification were often not in sync with hardware availability requirements to support in the training of the payload crew and to assist in procedural development.

4.1.1 Orientation training— The first exposure to orientation training, in the then-present reincarnation of the SL-4 experiments, was begun in September 1987 and was completed in February 1989. Training was accomplished at either ARC facilities or (for the jellyfish experiment) at the PI's lab. The crew received orientation to the ARC complement of rack-mounted hardware, i.e., RAHF, GPWS, and SMMI, the jellyfish experiment and associated hardware, and the mid-deck-stowed AEMs. The crew also received an orientation on the cardiovascular animals, which at this time were to be housed in an AEM. Interspersed within this window was a training session in May 1988 to review PCDT activities and associated tasks to be performed on a KC-135 flight in June 1988.

Approximately 47 orientation training hours were accomplished for each crew member during this interval of training. This figure does not include the additional hours each crew member spent prior to May 1988 nor the additional hours required to review training materials prior to the start of the scheduled training session.

4.1.2 Task training—During task training, the payload crew became proficient in all aspects of the experiment objectives through intensive and in-depth lectures on experiment unique hardware (EUH), stowed items, procedures, and "hands-on" training with specimens and available experiment hardware. Because of the overall launch schedule and the availability of the hardware and the crew, task training was often accomplished together with orientation training.

Task training on PCDT activities was provided on three training dates (September 1987, November 1987, and January 1989). The payload crew also received training on the jellyfish experiment, SMMI, GPWS, and RAHF. Approximately 49 hours (for each crew member) were accumulated in support of task training.

- 4.1.3 Phase training—Phase training was designed to allow the crew the opportunity to complete enough repetitions of the experiment so that (s)he would be able to complete the procedures at a defined level of time proficiency. Training was to have utilized the experiment operating procedures, payload specific hardware, and stowage items. This training opportunity was also designed to provide the crew with a level of proficiency that would guarantee a meaningful participation in the EVT. The crew logged approximately 37 hours each during this portion of the training, accomplished over a period of two years and three training opportunities.
- 4.1.4 Project integrated training—The objective of crew training during the SLS-1 EVT (February 28 to March 8, 1989) was to conduct project-integrated training of the payload crew members. They were to perform all ARC in-flight activities to assist in validation of the SLS-1 timeline. Although the crew were familiar with the ARC payload, this EVT was the first time they combined the tasks into operational procedures with most of the flight hardware and stowage items available for their use.

Unfortunately, the payload crew mission specialists were not available to support the EVT while the primes and backup payload specialists (PS) attended and

participated in a large number of the in-flight sessions. Their participation covered approximately 40 hours of the total 72-hour execute shift.

## 4.2 Mission Management Office Training

The objectives of the mission integrated training sessons (MITS) were twofold; they allowed the crew to develop their proficiency to a level of performance where they could successfully perform all the payload activities within the mission timeline, and they allowed the payload operations control center (POCC) cadre and payload experiment developer (PED) support the opportunity to rehearse in-flight ground protocols. MITS were similar to project integrated training, but included timeline performance of all mission experiments and other activities necessary to carry out the mission.

Each MITS occurred within a fully integrated Spacelab mockup and was supported by ARC training. Integration of the building 36 mockup began in June 1989. Confusion existed initially because ARC hardware was of mockup and not flight fidelity; the level of JSC building 36 QA was sometimes inappropriate. Training included not only nominal operations but also malfunction training.

The SLS-1 payload had the unique opportunity of participating in 10 simulations with the POCC cadre (including MMO and PED support personnel). In addition 5 joint integrated training simulations (JITS) were scheduled with the POCC cadre at MSFC, mission control personnel at JSC, and the crew traveling between the building 36 Spacelab mockup, the building 9 mid-deck mockup, and the building 5 simulators. Each of these training opportunities simulated different start and stop times on the overall mission timeline. This stipulation required that the mockup, including stowage, be configured to simulate the mockup as it would appear at the start time of the simulation for that particular FD.

Payload crew members participated in MIT. The alternate payload specialist supported all training simulations by serving as the voice interface between the crew and the POCC cadre. The orbiter crew were selected later than the payload crew and, therefore, their participation came later in the flow of these events. (Note: these additional assignments required that ARC provide orientation to the ARC payload as well as exposure to the hardware and in-depth training on any ARC experiments they were to perform in flight).

MITS dates and FDs simulated were as follows:

MITS#	Date	FD#
ı	July 26-27, 1989	1a
2	Aug. 23-25, 1989	2–4
3	Oct. 17-19, 1989	4–7
4	Dec. 5-8, 1989	2–5
5	Jan. 17–18, 1990	3–4
6	Mar. 12-16, 1990	4-6, 7-8
7	Apr. 17–19, 1990	1–3
8	Sept. 24-25, 1990	1
9	Nov. 26-28, 1990	4–5
10	Feb. 12, 1991	1

<sup>&</sup>lt;sup>a</sup>Spacelab activation.

JITS dates and FDs simulated were as follows:

JITS#	Date	FD#
Pre	Feb. 20-22, 1991	Simulation for POCC cadre only; alternate payload specialist
1	Mar. 20, 1991	] a
2	Apr. 2–3, 1991	4
3	Apr. 16-17, 1991	1–2
4	May 3, 1991	9ь

<sup>&</sup>lt;sup>a</sup>Ascent/activation.

#### 4.3 Lessons Learned

The following items address some of the difficulties associated with training a crew and demonstrate that in-flight operations should be given a higher level of priority during payload development and maturation. These "lessons learned" are presented from an operations standpoint. Delivery of the hardware to meet integration is highly critical, but it is the success or failure of the in-flight operations that will be remembered and used to determine the outcome of a mission. The following items address training and procedure development:

- The necessity that hardware be available to support procedure development and training conflicts with hardware verification and delivery dates to STS.
- Higher fidelity mockups of training hardware are required to support MITS.
- Spacelab mockups used to support MITS must be configured correctly and validated prior to the onset of this phase of training.
- Procedure development requires the use of highfidelity, flight-like hardware many months before the present payload-development schedule allows.

(Payload considered mature and frozen at CDR,  $\sim$ L - 18 months, but crew begins training between L - 24 and L - 18 months; consequently, procedure validation using flight-like hardware cannot occur early.)

- Month-by-month launch delays prolong the training program such that skills are dampened and performance quality decreases.
- Crew must be exposed to procedures that have been correctly formatted into a preliminary in-flight version at the onset of integrated training.
- Preliminary in-flight documentation must be available to support MIT.
- Clear and detailed science and engineering requirements that address crew operations covering the range of activities from photo/filming to in-flight data collection must be provided.
- Every activity timelined concurrently or on either side of an ARC experiment must be performed during a simulation.
- Possible stowage interference with other payload experiments must be determined when ARC experiments are performed.
- Changes to any procedures must be completed well in advance of L - 1 month. The MMO procedure delivery schedule must be changed to ensure that all procedure verification is done early in the documentation cycle.
- ARC must verify stowage and foam-fit checks while foam is in its locker, even though MMO is responsible for fabricating the foam.
- Pictures should be taken of hardware switch panels and stowage closeout for crew update/familiarization materials and for support of POCC in-flight activities.
- Individually wrapped items should be repackaged into groupings to avoid excessive garbage generation.
- SMMI weight kit needs to reworked, i.e., foam configuration must be tighter.
- Labeling of items should be as high a priority as the actual hardware concerns.
- Procedures sent to in-flight crew should always be in the same format. The ground should not be providing ground or MVAK procedures since the crew has probably never seen or worked with this version of the procedures. There should be only one source for the procedures.

<sup>&</sup>lt;sup>b</sup>Deorbit.

Greater details of lessons learned affecting PED elements were detailed in the ARC SLS-1 90-day report.

### 5.0 Science Results

# 5.1 Rodent Growth, Behavior, and Organ-Weight Changes Resulting from Spaceflight

**5.1.1 Introduction**— SLS-1 was dedicated to the study of responses of humans and rats to spaceflight and a period of reexposure to Earth's gravity. This first opportunity to perform detailed parallel studies on humans and rodents and studies on similarly treated rodents flown in two different types of habitats (individual and group housed) had these specific objectives:

- To verify the RAHF and AEM and the capability of these facilities to maintain healthy animals for experimental use.
- To compare changes in rats exposed to spaceflight to the changes seen at 1 g in rats housed in identical flight hardware and exposed to a similar flight environmental profile.
- To compare the changes seen in rats flown in the RAHF with those seen in rats flown in the AEM and to identify housing-related effects.
- To compare results from both human and rat experiments to determine whether the rat is a good model for the study of the effects of spaceflight on human physiology. (It should be noted that this objective is outside of the purview of this report and requires collaborative efforts between the human and animal subject investigators.)

**5.1.2 Methodology**– The following discussion of the procedures used in SLS-1 summarizes the more detailed account in the SLS-1 90-day report (AR-01449).

One hundred and sixty three male rats, (Rattus norvegicus, Sprague-Dawley strain, Taconic Farms) 30 days old were received at Hangar L, KSC, 28 days before the scheduled launch. A daily health check along with food, water, and body-weight measurements obtained every 3 days were used as selection criteria for the final pool of experimental animals. Flight-candidate rats were selected at L - 13 days, either grouped 5 per cage or individually housed, and were placed on flight food-bar diets (see Section 3.1.2, Flight Diet). Microbiological testing was performed at receipt and L-6 days in order to certify that the rodents were clear of organisms (per NASA Specific-Pathogen Free List, ref. 8). All flight-candidate rats were given preflight injections and blood draws for the hematology and bone-growth experiments (table 1). On L-2 flight rodents were selected randomly from a pool of candidates displaying good general health, normal

growth curves, and normal food- and water-consumption rates. Observations of their behavior during hematology and bone-growth experiment procedures also helped determine the final flight-candidate pool.

Twenty-nine flight rats were loaded into the flight cages approximately 33 hours before launch. Nineteen rats were flown in individual cages in the RAHF, while 10 rats were flown, 5 rats each, in two AEMs. Real-time control rats were maintained throughout the flight period: RAHF control animals in vivarium cages and AEM control animals in flight-qualified AEMs. The total shuttle flight lasted 9 days, 3 hours, 13 minutes. Except for two rats, which were briefly handled by the crew, the flight rats remained in their habitats throughout flight. Flight animals had access to food bars and water ad libitum. In addition, on FD 8 (L + 7), all RAHF rats also received 2 to 3 Gel Paks, each containing 30 ml of 1-percent agar solution as a water supplement.

Beginning at about three hours after launch (L+0), single and group house control rats were dissected and organ weights and tissue samples were taken for the biospecimen sharing program (BSP). Whole organ weights were obtained for spleen, heart, thymus, adrenals, kidneys, liver, and testes.

On L + 2 days the science team and the ground control rodents were flown from KSC to the DFRC payload receiving facility (PRF) at Edwards Air Force Base, California. Before being loaded into the passenger compartment of the aircraft, the singly housed rodents were weighed and transferred from their vivarium cages to compartmentalized, rodent-shipping containers; food and water were available ad libitum. On the other hand, AEM-housed animals were not removed from their habitat and the lighting and fan systems were powered by batteries for the flight from Florida to California. On landing at the PRF, the ground control rodents that had been transported in shipping cages were examined for possible injury or illness enroute. They were placed in clean vivarium cages with fresh food and water. Routine maintenance was resumed for all animals held in animal holding rooms for the duration of the experiment.

Upon landing (R+0), flight and control rats were removed from their habitats, weighed, and checked by the ARC veterinarian for general health. All rats were photographed and videotaped. After this initial processing, half of the flight animals designated as the R+0 flight group and their ground control complement were dissected while the remaining rats underwent injections and blood draws in support of the bone and hematology experiments. The residual groups were maintained throughout the recovery period in vivarium cages with

food and water ad libitum and were dissected after 9 days, a recovery period equal to the mission length (R + ML).

A DFPT simulating the profile of environmental conditions in Spacelab during the SLS-1 spaceflight was conducted at KSC using flight RAHF and flight AEM hardware. The DFPT beginning with the receipt of rats on June 6, 1991, mirrored the mission timeline and matched the temperature, humidity, and light/dark cycles experienced by the rodents during flight. Significant operational events (Gel Pak additions, hardware maintenance, and rodent handling) were also repeated; however, the airplane trip to California was not duplicated for the DFPT ground control rats, nor were the g force, vibration, and noise profiles experienced during spaceflight by the animals held in the RAHF and AEMs. All DFPT dissection operations were performed per the flight procedures at launch (L + 0), landing (R + 0) and after a recovery of 9 days (R + ML). The fidelity of the repetition of the DFPT procedures to those of the mission can be most easily seen in the rodent chronology (table 1).

Statistical analyses on a Macintosh computer utilized Statview II and SuperAnova software programs (Abacus Concepts, Berkeley, Calif.) in a 2 × 2 × 3 fashion (Flight/DFPT; AEM/RAHF; preflight/flight/recovery). Organ-weight data were contrasted via analysis of variance (ANOVA) with Bon Ferroni corrections for multiple groups (tables 2 and 3). Food, water, and body-weight data were contrasted via repeated measure ANOVA for specific time windows (tables 4 and 5).

#### 5.1.3 Results-

General rodent health and behavior: The rats remained healthy during all phases of the mission and DFPTs, as confirmed by observations made by the flight crew and the ARC veterinarian. However, the flight crew did express a concern that the particulates floating in the AEMs during spaceflight might have been aspirated by the rats. As noted in Section 3.3, Anomalies, no unusual pathology was found in the lungs of the exposed animals.

On landing, flight rats appeared weak and shaky with a loss of muscle tone, and they moved as if their joints and muscles hurt. They felt soft-bodied when handled. They were lethargic and less inquisitive than real-time 1-g controls. Flight rats exhibited reduced use of their tails as stabilizing tools, and displayed difficulty in balancing themselves on their hindlimbs in an upright posture; effects were more pronounced in AEM rats than in RAHF rats. These changes were markedly reduced in the rodents by the second postflight day. No differences in behavior were distinguishable between groups by the third post-

flight day. Rodent health remained good throughout the recovery period.

Body weight and weight gain: Body weights were not different between mission and DFPT flight groups at loading (mission =  $285.3 \pm 3.1$  g, DFPT =  $281.8 \pm 3.1$  g; p < 0.05 percent). Mission flight rats gained significantly less body weight during the flight period than DFPT flight rats  $(4.2 \pm 0.2 \text{ vs. } 6.0 \pm 0.2 \text{ g/day}, p < 0.0001)$ . As a result, mission flight rats weighed significantly less at unloading (331.0  $\pm$  3.5 g vs. 347.4  $\pm$  3.7 g, p < 0.01). The mission flight rats lost  $6.9 \pm 0.7$  g/day for the first two days after landing compared to a net gain of  $2.9 \pm 0.5$  g/day in the DFPT flight rats (p < 0.0001). Weight gain after this initial "trough" (fig. 11(a)) was not different from that of DFPT flight rats; however, body weights of mission flight animals remained significantly below those of the DFPT flight rats throughout recovery (p < 0.0001). All rat groups except the untreated baseline control animals showed a marked weight loss from R + 8 to R + 9. There is no difference in body weights between flight RAHF and flight AEM rats on any day during the recovery period.

**Food:** Daily food consumption (indexed per 100 g of body weight) was greater during the flight period in mission flight rats than in DFPT flight rats  $(9.4 \pm 0.2 \text{ g vs.} 8.7 \pm 0.1 \text{ g/day}, p < 0.01)$  (fig. 11(b)). The mission flight rats significantly decreased their daily food consumption upon landing, consuming significantly less than the DFPT flight rats during the first two days of recovery (-41 percent vs. -2 percent, p < 0.001). The average daily food consumption for the mission flight rats was 28 percent less than for the DFPT flight rats throughout the postflight recovery period (p < 0.0001).

Water: In-flight water utilization differences between mission flight and DFPT flight rats were insignificant (fig. 11(c)). Mean water utilization for mission flight and DFPT flight AEM rats combined (N = 20)was greater when compared to mission flight and DFPT flight RAHF rats combined (N = 38) (44.1 vs. 27.5 ml/day, p < 0.0001). Mean water utilization for combined AEM rats dropped 50 percent from the in-flight mean during the first two days of recovery, while mission flight and DFPT flight RAHF rats increased utilization 23 percent and 7 percent, respectively, for the same period (fig. 11(d)). Daily water-utilization rate after R + 3 days was not different between flight RAHF and flight AEM rats, but was 42 percent greater (indexed to body weight) in mission flight AEM and RAHF rats combined than DFPT flight AEM and RAHF rats combined (p < 0.01).

Table 1. SLS-1 Mission and DFPT Rodent Chronology

			MISSION		DFPT	Time	Event or procedure
lission day	Elapsed	Flight day	Date	Time	Date		
	day 0		5/08/91		6/12/91	R	eceipt of launch contingency group 2 at Hangar L KSC; age 30 $\pm$ 3 days; microbiology sampling
L - 28	1		5/09/91		6/13/91		
L - 27			5/10/91	1 (	6/14/91		
L-26	2		5/11/91		6/15/91	F	ood, water, and body weight; health check
L-25	3		5/12/91		6/16/91		
L - 24	4				6/17/91		
L-23	5		5/13/91		6/18/91	F	ood, water, and body weight; health check
L-22	6		5/14/91		6/19/91		
L-21	7		5/15/91		6/20/91		
L-20	8		5/16/91	9	6/21/91	F	ood, water, and body weight; health check
L – 19	9		5/17/91	1	6/22/91		
L – 18	10		5/18/91		6/23/91		
L – 17	11		5/19/91		6/24/91	F	Food, water, and body weight; health check
L – 16	12		5/20/91		6/25/91		
L – 15	13		5/21/91		6/26/91		
L-14	14		5/22/91		6/27/91	1	Flight candidates selected; group or singly housed; diet switched to food bars
L – 13	15		5/23/91	ļ	0/2/191		All groups: Sub-cue, Calcein bone marker; IV 200–250-ml blood draw
			7/04/04	ļ	6/28/91		
L - 12	16		5/24/91		6/29/91		
L – 11	17		5/25/91		6/30/91		
L - 10	18		5/26/91		7/01/91		
L-09	19		5/27/91		7/01/91		R + ML: Inject IV: 51Cr-RBC, 125I-albumin, 0.9-percent saline; IV 200-250-ml blood draw
L - 08	20		5/28/91		7/02/91		R + 0: Inject IV: 51Cr RBC, 0.9-percent saline: IV 200-250-ml blood draw
L - 07	21		5/29/91		7/03/91		R + ML: IV 150-mi blood draw
			ı	4	7/04/01		R + 0 group: IV 150-ml blood draw
L-06	22		5/30/91	ł	7/04/91		All groups: Inject Sub-cue, demeclocycline; microbiology sampling
				İ	7/05/91		, g
L - 05	23		5/31/91				
L - 04	24		6/01/91		7/06/91		
L - 03	25		6/02/91	i	7/07/91		All groups: Sub-cue, demeclocycline
L - 02	26		6/03/91		7/08/91		Flight, redents collected from candidate pool; loaded RAHF cages at L = 33 nr; at pad 39A L =
							Flight and ground control rodents loaded into AEMs; AEMs and jellyfish at pad 39A L - 17 hr
L - 01	27		6/04/91		7/09/91		SLS-1 (STS 40) launched
L+0	28	1	6/05/91	9:25 a.m. EDT	7/10/91	FDT	L + 0: Start dissect group housed #1-5 (AEM control)
L+0			ł	12:15 p.m. EDT		12:30 p.m. EDT	L + 0: Start dissect group housed #6–15 (RAHF control)
L+0			1	2:00 p.m. EDT		2:00 p.m. ED1	L + U: Start dissect singly housed we have the
L+01	29	2	6/06/91	ļ	7/11/91		Mission: ground controls flown to DFRC PRF
L+02	30	3	6/07/91		7/12/91		Mission: ground controls now to 51 to 51
L+03	31	4	6/08/91		7/13/91		
L + 04	32	5	6/09/91		7/14/91		
L+05	33	6	6/10/91	İ	7/15/91		
L+06	34	7	6/11/91		7/16/91		All RAHF animals received 2-3 Gel Paks containing 30 ml 1-percent agar as water supplemental sup
L + 07	36	8	6/12/91		7/17/91		All RAME animals received 2-3 del 1 axs containing de this profit
L+08	36	9	6/13/91	į	7/18/91		DEDO DEEL Weeks divertion: 9 d 3 hr 13 min
R+0	37	10	6/14/91	8:38 a.m. PDT	7/19/91		SLS-1 landed at DFRC PRF; flight duration: 9 d, 3 hr, 13 min
n+0	O,		1	9:15 a.m. PDT		12:15 p.m. EDT	R + 0: Start dissect AEM ground control #16-20
				10:55 a.m. PDT		1:30 p.m. EDT	R + 0: Start dissect AEM flight #21-25
				12:55 p.m. PDT		3:35 p.m. EDT	R + 0: Start dissect RAHF flight #26-35
			1	4:10 p.m. PDT		6:55 p.m. EDT	R + 0: Start dissect RAHF ground control #36-45
			1				R + ML: Inject IV: 51Cr-RBC, 125I-albumin, 59Fe citrate; 200–250-ml blood draw
			1				R + ML: inject_IP: calcein/3H-proline in 0.9-percent saline
	~		6/15/91		7/20/91		R + ML: IV 150-ml blood draw
R+01	38		6/16/91		7/21/91		
R+02	39		6/17/91		7/22/91		R + ML: IV 150-ml blood draw
R + 03	40		6/18/91		7/23/91		R + ML: IV 150-ml blood draw
R + 04	41		6/19/91		7/24/91		
R + 05	42		ı		7/25/91		
R + 06	43		6/20/91		7/26/91		
R + 07	44		6/21/91		7/27/91		R + ML: inject IV 51Cr-RBC, 125I-albumin, 0.9-percent saline; IV 200-250-ml blood draw
FI + 08	45		6/22/91		7/28/91		B + ML; IV 3.0-ml blood draw
R + 09	46		6/23/91	0.45 DDT	L	12:15 nm ED	T R + ML: Start dissect AEM ground control #46-50
			1	9:15 a.m. PDT		1:30 nm ED1	R + ML: Start dissect AEM flight #51-55
			1	10:55 a.m. PDT		3:35 nm ED	T R + ML: Start dissect RAHF flight #56-65
			1	12:55 p.m. PDT		6.55 nm ED	r D . Mt · Start dissect RAHF ground control #66-75
			1	4:10 p.m. PDT	1	6.55 p.m. EU	Dissection: untreated group housed #200-204; untreated singly housed #205-214
			6/24/91	•	7/29/91		Dissection: untreated group housed #200-204; untreated singly housed #200-2

Table 2. Organ weights a flight vs. delayed flight profile test controls at launch, recovery, and recovery + mission length (9 days)

Organ		L + 0 (g)	Percent ∆ Flight	R + 0 (g)	Percent Δ Recovery	R + ML (g)
Spleen	Flight	1.01 ± 0.03	-19.5 ± 0.31 <sup>b</sup>	0.82 ± 0.03 <sup>c</sup>	0.0 ± 4.5	0.82 ± 0.04
	DFPT	$0.96 \pm 0.04$	$0.54 \pm 0.23$	$1.00 \pm 0.03$	$-7.3 \pm 3.6$	0.90 ± 0.04
Indexed spleen	Flight	0.34 ± 0.01	-28.21 ± 2.3 <sup>d</sup>	0.25 ± 0.01 <sup>b</sup>	-1.2 ± 4.5 <sup>b</sup>	0.24 ± 0.01
	DFPT	$0.32 \pm 0.01$	-11.27 ± 2.7	$0.29 \pm 0.01$	$-18.5 \pm 3.1$	$0.23 \pm 0.01$
Heart	Flight	1.17 ± 0.04	-1.37 ± 2.2	1.16 ± 0.02 <sup>e</sup>	1.3 ± 2.1 <sup>e</sup>	1.17 ± 0.02 <sup>c</sup>
	DFPT	$1.13\pm0.03$	8.5 ± 1.8	1.22 ± 0.02	8.9 ± 2.5	1.32 ± 0.03
Indexed heart	Flight	$0.40 \pm 0.01$	-11.65 ± 1.5 <sup>b</sup>	$0.35 \pm 0.004$	-0.3 ± 1.2	0.35 ± 0.004
	DFPT	$0.38 \pm 0.01$	-4.51 ± 1.8	0.36 ± 0.01	$-3.7 \pm 3.2$	0.35 ± 0.004
Liver	Flight	12.98 ± 0.24	3.9 ± 2.2	13.49 ± 0.29	-16.8 ± 1.7 <sup>d</sup>	11.23 ± 0.021 <sup>d</sup>
	DFPT	$12.63 \pm 0.32$	5.7 ± 1.7	13.36 ± 0.21	$0.0 \pm 2.8$	$13.36 \pm 0.34$
Indexed liver	Flight	4.40 ± 0.07	-7.13 ± 1.3	4.08 ± 0.06	~17.9 ± 1.7 <sup>e</sup>	3.35 ± 0.06
	DFPT	$4.24 \pm 0.06$	$-6.79 \pm 2.9$	3.95 ± 0.06	$-12.0 \pm 2.0$	$3.50 \pm 0.08$
Thymus	Flight	$0.88 \pm 0.04$	-22.0 ± 4.2 <sup>e</sup>	0.68 ± 0.04	-8.6 ± 4.3	0.62 ± 0.03
	DFPT	$0.83 \pm 0.03$	$-10.4 \pm 2.9$	$0.75 \pm 0.02$	-13.7 ± 5.4	$0.64 \pm 0.04$
Indexed thymus	Flight	0.30 ± 0.02	-30.65 ± 3.4 <sup>e</sup>	0.21 ± 0.01	-9.5 ± 4.3 <sup>e</sup>	0.19 ± 0.01
	DFPT	$0.28 \pm 0.01$	-21.26 ± 2.4	0.22 ± 0.01	-24.3 ± 4.1	0.17 ± 0.01
Kidney (total)	Flight	2.32 ± 0.05	10.2 ± 2.3	$2.56 \pm 0.06$	-9.7 ± 1.5 °	2.31 ± 0.04 <sup>b</sup>
	DFPT	$2.30 \pm 0.05$	12.9 ± 1.9	$2.60 \pm 0.05$	1.1 ± 2.6	2.64 ± 0.09
ndexed total kidney	Flight	0.79 ± 0.01	$-1.36 \pm 1.3$	0.78 ± 0.01	-10.9 ± 0.9	0.67 ± 0.01
	DFPT	$0.77 \pm 0.01$	$-0.59 \pm 1.4$	0.77 ± 0.02	-10.0 ± 2.1	$0.67 \pm 0.01$ $0.69 \pm 0.02$
Testis (total)	Flight	$3.05 \pm 0.08$	4.9 ± 1.9 <sup>e</sup>	3.19 ± 0.06	4.7 ± 1.7	3.34 ± 0.05 <sup>b</sup>
	DFPT	2.94 ± 0.07	11.8 ± 2.4	$3.28 \pm 0.07$	9.9 ± 2.5	3.61 ± 0.08
ndexed total testis	Flight	1.03 ± 0.03	-6.13 ± 1.5	0.97 ± 0.02	3.3 ± 2.4	1.0 ± 0.02
	DFPT	$0.99 \pm 0.02$	$-1.67 \pm 2.0$	$0.97 \pm 0.02$	-3.0 ± 2.0	$0.94 \pm 0.02$
Adrenal (total)	Flight	0.0385 ± 0.0012	9.9 ± 5.3	0.0423 ± 0.0021	9.5 ± 3.8	0.0460 ± 0.0016
. ,	DFPT	0.0396 ± 0.0007	$5.4 \pm 3.1$	$0.0418 \pm 0.0014$	15.9 ± 4.5	$0.0480 \pm 0.0018$ $0.0482 \pm 0.0024$
ndexed total adrenal	Flight	0.0131 ± 0.0004	-1.99 ± 4.40	0.0128 ± 0.0006	$8.5 \pm 4.4$	
	DFPT	$0.0133 \pm 0.0003$	-7.57 ± 2.90	$0.0120 \pm 0.0000$ $0.0124 \pm 0.0004$	$6.3 \pm 4.4$ $2.2 \pm 4.3$	$0.0138 \pm 0.0006$ $0.0126 \pm 0.0006$
N for period	Flight	15 basal	15	15	14	
•	DFPT	15 basal	15	15	13	14 13

<sup>&</sup>lt;sup>a</sup>All weights are in grams. Indexed weights are in grams/100 grams of body weight. All numbers are mean ± SE.

b p < 0.01

c p < 0.001 d p < 0.0001 e p < 0.05

Table 3. Organ weights a: flight Research Animal Holding Facility vs. flight animal enclosure module: launch, recovery, and recovery + mission length (9 days)

Organ		L + 0 (g)	Percent ∆ Flight	R + 0 (g)	Percent Δ Recovery	R + ML (g)
Spleen	RAHF	1.01 ± 0.38	-18.4 ± 3.3	0.82 ± 0.03	$3.6 \pm 5.8$	$0.85\pm0.05$
	AEM	$1.03 \pm 0.03$	21.9 ± 7.1	$0.80\pm0.07$	$-6.4 \pm 6.8$	$0.75 \pm 0.05$
ndexed spleen	RAHF	0.35 ± 0.01	-27.3 ± 2.8	0.25 ± 0.10	3.3 ± 6.2	$0.26 \pm 0.02$
ndexed spiceri	AEM	$0.34 \pm 0.004$	-30.1 ± 4.2	$0.24 \pm 0.01$	$-9.3 \pm 4.7$	$0.22 \pm 0.01$
Heart	RAHF	1.13 ± 0.04	1.9 ± 2.6 <sup>e</sup>	1.15 ± 0.03	$0.1 \pm 2.6$	$1.16 \pm 0.03$
rieart	AEM	1.26 ± 0.10	$-7.8 \pm 1.9$	$1.16 \pm 0.02$	$3.5 \pm 3.9$	$1.20 \pm 0.05$
Indexed heart	RAHF	0.39 ± 0.01	-9.1 ± 1.5 <sup>b</sup>	0.35 ± 0.01	-0.3 ± 1.1	$0.35 \pm 0.004$
illuexed fleat	AEM	$0.42 \pm 0.03$	$-16.7 \pm 1.7$	0.35 ± 0.01	$-0.3 \pm 2.9$	$0.35 \pm 0.01$
Liver	RAHF	12.92 ± 0.28	3.0 ± 2.9	13.30 ± 0.37	-14.5 ± 1.8	$11.37 \pm 0.24$
Livei	AEM	13.11 ± 0.47	$5.8 \pm 3.4$	13.36 ± 0.21	$0.0 \pm 2.8$	$13.36 \pm 0.34$
Indexed liver	RAHF	4.40 ± 0.01	-8.3 ± 1.8	4.04 ± 0.08	-14.7 ± 1.6 <sup>b</sup>	$3.45 \pm 0.06^{e}$
maexea liver	AEM	$4.39 \pm 0.16$	$-4.8 \pm 0.9$	4.17 ± 0.04	$-23.8 \pm 1.8$	$3.18 \pm 0.08$
Thumus	RAHF	0.85 ± 0.05	-22.0 ± 4.9	0.66 ± 0.04	–1.1 ± 3.8 <sup>e</sup>	0.66 ± 0.02
Thymus	AEM	0.93 ± 0.08	$-21.8 \pm 8.7$	$0.73 \pm 0.08$	$-22.0 \pm 7.1$	$0.57 \pm 0.05$
Indoved thumis	RAHF	0.29 ± 0.02	-30.9 ± 4.0	0.20 ± 1.2	-1.5 ± 2.6 <sup>b</sup>	0.20 ± 0.01 <sup>e</sup>
Indexed thymus	AEM	0.31 ± 0.03	-30.2 ± 7.1	0.22 ± 0.01	-24.1 ± 7.7	$0.17 \pm 0.02$
Kidney (total)	RAHF	2.37 ± 0.06	11.5 ± 2.5	2.65 ± 0.06	-11.5 ± 1.9	2.34 ± 0.05
Kidney (total)	AEM	$2.23 \pm 0.10$	$7.8 \pm 5.3$	$2.40 \pm 0.12$	$-6.5 \pm 1.7$	$2.24 \pm 0.04$
Indexed total kidney	RAHF	0.81 ± 0.01 <sup>e</sup>	-1.0 ± 1.6	0.80 ± 0.01 <sup>b</sup>	-11.79 ± 1.3	0.71 ± 0.01 <sup>b</sup>
Indexed total kidney	AEM	$0.74 \pm 0.03$	$-3.0 \pm 3.4$	$0.72 \pm 0.02$	-9.5 ± 0.9	$0.65 \pm 0.01$
Testis (total)	RAHF	3.10 ± 0.08	4.0 ± 2.4	3.22 ± 0.08	4.5 ± 1.5	$3.37 \pm 0.05$
Testis (total)	AEM	$2.95 \pm 0.21$	$6.6 \pm 3.0$	$3.14 \pm 0.09$	$4.8 \pm 4.1$	$3.29 \pm 0.13$
indexed total testis	RAHF	1.06 ± 0.02	-7.2 ± 2.1	0.98 ± 0.02	$4.4 \pm 2.8$	1.02 ± 0.03
ilidexed total testis	AEM	$0.98 \pm 0.07$	-3.9 ± 2.1	$0.95 \pm 0.02$	$-1.3 \pm 4.6$	$0.96 \pm 0.04$
Adropal (total)	RAHF	0.0388 ± 0.0013	15.2 ± 7.1	0.0447 ± 0.0028	$6.7 \pm 4.6$	0.0477 ± 0.002
Adrenal (total)	AEM	$0.0338 \pm 0.0016$ $0.0378 \pm 0.0026$	$-1.0 \pm 4.9$	0.0376 ± .00018	$14.6 \pm 6.6$	0.0431 ± 0.002
Indexed total advence	RAHF	0.0133 ± 0.0005	2.3 ± 6.1	0.0136 ± 0.0008	6.8 ± 5.8	0.0145 ± 0.000
Indexed total adrenal	AEM	$0.0126 \pm 0.0008$	$-10.5 \pm 2.8$	0.0113 ± 0.0004	11.5 ± 7.4	0.0126 ± 0.000
N for pariod	RAHF	10 basal	10	10	9	9
N for period	AEM	5 basal	5	5	5	5

 $<sup>^</sup>a$  All weights are in grams. Indexed weights are in grams/100 grams of body weight. All numbers are mean  $\pm$  SE  $^b$  p < 0.01  $^c$  p < 0.001 d p < 0.0001  $^e$  p < 0.05

Table 4. Food and water utilization/body weights a: Flight rats vs. delayed flight profile test rats

		Flight	Percent ∆ from	Early recovery	Percent Δ from	Late recovery
		L - 2 to R + 0	flight to recovery	R+1 to R+2	early to late recovery	R+3 to R+9
Mean body weight	Flight	300.2 ± 3.1 <sup>b</sup>	6.5	319.6 ± 5.4 <sup>d</sup>	3.3	330.3 ± 5.1 <sup>d</sup>
	DFPT	314.6 ± 3.1	13.7	$357.7 \pm 5.5$	4.6	$374.3 \pm 6.0$
Daily weight gain	Flight	4.2 ± 2 <sup>d</sup>		-6.9 ± 0.7 <sup>d</sup>		2.7 ± 0.2
	DFPT	$6.0 \pm 0.2$		$2.9\pm0.5$		2.6 ± 0.2
Food	Flight	$28.0 \pm 0.6$	-40.7	16.6 ± 0.9 <sup>c</sup>	18.1	19.5 ± 0.5 <sup>d</sup>
	DFPT	$27.6 \pm 0.4$	-2.2	27.1 ± 0.7	1.1	$27.3 \pm 0.6$
ndexed food	Flight	$9.4 \pm 0.2^{b}$	-44.7	5.24 ± 0.6 <sup>b</sup>	15.4	6.0 ± 0.1 <sup>d</sup>
	DFPT	$8.8 \pm 0.1$	-14.8	7.51 ± 0.2	-4	$7.2 \pm 0.1$
Vater	Flight	$30.3 \pm 1.7$	12.9	33.2 ± 2.8	11.1	36.9 ± 2.2
	DFPT	$29.5 \pm 1.8$	-2.4	28.1 ± 3.4	7.1	30.1 ± 2.9
ndexed water	Flight	$9.8 \pm 0.5$	7.1	10.5 ± 0.9 <sup>e</sup>	7.6	11.3 ± 0.7 <sup>b</sup>
	DFPT	$9.1 \pm 0.6$	-23.1	7.0 ± 1.3	14.3	$8.00 \pm 0.8$

<sup>&</sup>lt;sup>a</sup>All weights are in grams. Indexed weights are in grams/100 grams of body weight. Indexed water weights are in ml/100 grams of body weight. Numbers are mean ± SE.

Table 5. Food and water utilization/body weights a: research animal holding facility rats vs. animal enclosure module rats

		Flight L – 2 to R + 0	Percent Δ from flight to recovery	Early recovery R + 1 to R + 2	Percent $\Delta$ from early to late recovery	Late recovery R + 3 to R + 9
Mean body weight	RAHF	$298.3 \pm 3.4$	5.6	315.1 ± 5.5 <sup>d</sup>	3.1	325.0 ± 6.1
	AEM	$303.7 \pm 6.4$	7.9	$327.8 \pm 8.1$	3.7	339.9 ± 8.3
Daily weight gain	RAHF	$4.0 \pm 0.2$	_	-7.2 ± 1.0	_	2.6 ± 0.2
	AEM	$4.4 \pm 0.3$	_	$-6.3 \pm 0.6$	<del></del>	$2.7 \pm 0.1$
Food	RAHF	28.1 ± 0.7	-39.9	16.9 ± 2.1	16	19.6 ± 0.5
	AEM	$27.3 \pm 0.3$	-49.8	13.7 ± NA	38	18.9 ± NA
Indexed food	RAHF	$9.4 \pm 0.2$	-42.6	$5.4 \pm 0.6$	11.1	6.0 ± 0.1
	AEM	$9.0 \pm 0.1$	-53.3	4.2 ± NA	33.3	5.6 ± NA
Water	RAHF	$29.2 \pm 1.6$	18.9	34.6 ± 2.6	6.9	37.0 ± 2.4
	AEM	$40.5 \pm 2.9$	-49.6	$20.4 \pm NA$	78.9	36.5 ± NA
Indexed water	RAHF	9.4 ± 0.5 <sup>e</sup>	17	11.0 ± 0.8	3.6	11.4 ± 0.7
	AEM	$13.3 \pm 1.0$	-53.4	$6.2 \pm NA$	72.6	10.7 ± NA

<sup>&</sup>lt;sup>a</sup>All weights are in grams. Indexed weights are in grams/100 grams of body weight. Indexed water weights are in ml/100 grams of body weight. Numbers are mean ± SE.

b p < 0.01

 $<sup>^{\</sup>circ}$  p < 0.001

 $<sup>^{\</sup>rm d}$  p < 0.0001

e p < 0.05

 $<sup>^{</sup>b}$  p < 0.01

 $<sup>^{\</sup>circ}$  p < 0.001

<sup>&</sup>lt;sup>d</sup> p < 0.0001

e p < 0.05

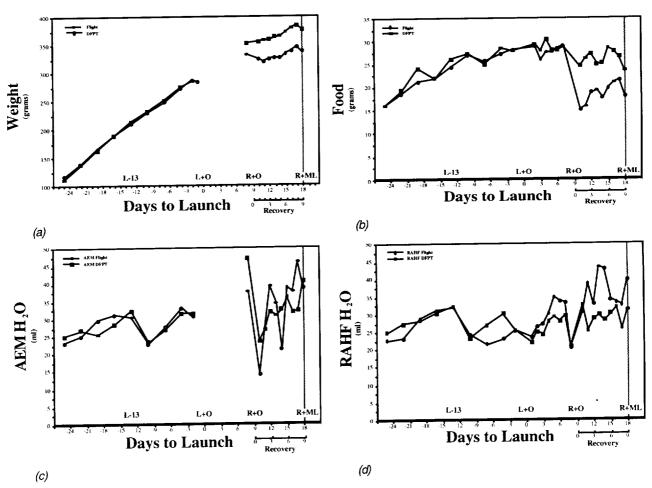


Figure 11. Comparative AEM and RAHF flight and DFPT data.

Organ weights: Mission flight rats exhibited 20 percent less spleen weight than preflight controls and significantly less than DFPT flight rats at landing (0.82 g vs. 0.97 g, p < 0.001). Heart weights of mission flight rats were 1 percent less than those of preflight controls and significantly less than DFPT controls on landing day (1.16 g vs. 1.22 g, p < 0.05). Total indexed kidney weight was greater in mission RAHF rats than mission AEM rats at landing day (0.80 g/100 g vs. 0.72 g/100 g, p < 0.01). No other organ differences were seen between RAHF and AEM rats at landing. After recovery, mission flight RAHF rats showed greater indexed liver weight (3.45 g/100 g vs. 3.18 g/100 g, p < 0.05) and greater indexed thymus weight (0.20 g/100g vs. 0.17 g/100 g, p < 0.05) compared to flight AEM rats.

**5.1.4 Discussion/conclusions**— Spaceflight results in significant decreases in body weight gain and, without concomitant reduction in food consumption, results in greater caloric intake per body weight. Reexposure to 1 g results

in dramatic reduction in body weight and in food intake on R+0 and R+1 (fig. 11(a)). These changes appear reversed after two days of recovery, although reduced body weight and appetite remain. The extreme decrease in food consumption may be indicative of decreased caloric requirements, or possibly of dehydration, but this is not clear.

Behaviorally, rodents return from spaceflight not unlike humans, with reduced muscle tone and altered coordination. In addition, rodents appear to require time to find their "land legs." This lethargy was reported in reference 9. Videotaped data from SLS-1 underscores the need to perform more quantitative biomechanical studies of rodents during flight and recovery, especially during the first two recovery days.

SLS-1 RAHF rats increased their water consumption in flight up to the fifth day (fig. 11(c)), at which point their water intake was significantly greater than that of RAHF rats (p < 0.05). This gradual increase in water use is

similar to that seen on SL-3 (unpublished data), although SLS-1 data suggest that this may be a transient increase that reverses by the sixth FD. The addition of water supplements (Gel Paks) to RAHF cages on FD 8 caused measured water consumption to drop markedly on the final FD and may have obscured water-consumption data from early recovery. This situation may help explain the striking difference in water consumption between RAHF and AEM rats upon return to 1 g. In addition, the marked drop in habitat temperature between AEMs and vivarium cages (5–8°C) on landing day probably contributed to the dramatic reduction in water-consumption rate for AEM rats.

The increase in water consumption by flight rats during the period from R+3 days to the final dissection on R+9 days may be related to dehydration from space-flight, although volume reloading in response to successive postflight blood draws cannot be ruled out. In addition, food consumption and weight gain decreased markedly from R+8 to R+9. These changes are seen in all rats and as such are likely a response to extensive handling on R+8 and are probably not associated with recovery from spaceflight.

The differences in water consumption between space-flown AEM rats and RAHF rats (fig. 11(c) and 11(d)) are similar to the differences seen between group and single housed controls in an Earth-gravity environment. The greater water utilization by group housed rats is at least in part due to greater ambient temperature of the group habitat (3–5°C), whether it be an AEM or vivarium cage. This greater habitat temperature is partially due to increased total body heat and in the AEM seems to be accentuated by the radiant heat from incandescent lighting. The marked increase in water utilization by AEM rats may be also confounded by inadvertent lixit activation as the "weightless" rats float and brush up against the water source.

The decreases in spleen mass seen in SLS-1 rats agree with data reported in references 10 to 12. The results from SL-3 and Cosmos identify decreases in spleen weight of 20–24 percent and 13 percent, respectively. Additional data from the combined hematology studies on SLS-1 should shed more light on this phenomenon. Data from R + ML spleen weights indicate that flight spleen weight remained unchanged throughout the recovery period, whereas, curiously, the spleen weight of DFPT rats decreased 8 percent during this period (possibly in response to blood-draw protocols). The decrease in heart mass seen in SLS-1 rats after spaceflight may reflect cardiovascular deconditioning identified in human subjects and previously summarized (ref. 13).

At R + ML the smaller absolute weight of flight rat heart, liver, kidney, and testes may be indicative of stress during recovery, although it may simply reflect the smaller body weight of rats at this point. The differences in indexed liver, kidney, and thymus weights seen between RAHF and AEM rats at R + ML are probably associated with a caging phenomenon. Individually housed rats are known to manifest signs of isolation stress phenomena, which can affect organ weights as well as cause changes in hepatic enzyme levels (ref. 14). Stress from other environmental factors, including heat and vibration, has been shown to alter kidney weight (ref. 15). It is important, therefore, to distinguish the physiological changes resulting from microgravity from those caused by environmental and operational perturbations.

In conclusion, the RAHF and AEM have both proved to be valuable habitats for the maintenance of rats as experimental subjects during spaceflight. Minor anomalies include temperature regulation within the AEM, which is currently being addressed by the SLSPO at ARC.

The results of SLS-1 indicate that the rat serves as a valuable model for basic research regarding the effects of spaceflight as well as a useful surrogate for certain human studies. Detailed investigations of the organs weighed in this study and other tissues obtained from rats during SLS-1 will help define the limitations of this model.

# **5.2 Spacelab Life Sciences Experiments: ARC SLS-1 Experiments**

Summaries of Results— The original SL-4 experiment payload, which was renamed SLS-1, was very different by flight from its first EVT in 1985: Monkeys and instrumented rats were eliminated. By flight, seven of the original experiments remained and the jellyfish experiment was added along with a BSP.

Given the final 1991 launch date for SLS-1 and the limitations of activities, the flight nonetheless served as an excellent control for the comparison of animals flown under two different animal maintenance systems in the microgravity environment, the RAHF and the AEM. The former provided for single animal housing and a controllable environment; the latter gang-caged animals in a unit that was greatly dependent on the outside environment for internal-cage temperature control. In addition, monitoring of individual feeding and watering was not available in the AEMs, as it was in the RAHF. Data gathered from SLS-1 were the control to the next dedicated Life Sciences mission, SLS-2. SLS-1 was the only opportunity to immediately perform ground controls in backup flight hardware.

Abstracts submitted by the primary investigators and their co-investigators follow; the abstracts summarize the data gathered from the SLS-1-mission-associated activities.

# Experiment 012: Regulation of Erythropoiesis in Rats during Spaceflight

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Spaceflight anemia (decrease of red blood cell (RBC) numbers in the circulating blood) has been documented since Gemini flights of the early 1960s. The objective of the present experiments was to aid in answering the question: What mechanism(s) is responsible for the anemia experienced by astronauts? In experiment 012, measurements were made of the various factors affecting erythropoiesis in a group of rats and their appropriate controls.

#### Objectives:

- Determine if any changes in serum erythropoietin
   (Epo) levels occurred in rats exposed to microgravity.
- Determine if there were any changes in standard hematological parameters: hemoglobin (Hgb), hematocrit (Hct), RBC count, platelet count, reticulocyte count, white blood cell (WBC) count, and WBC differential count.
- Enumerate lymphocyte subsets in peripheral blood.
- Determine the effect of weightlessness on the responsiveness of erythropoietin-sensitive bone marrow cells (burst forming unit-erythroid (BFU-e) and colony forming unit-erythroid (CFU-e) in vitro cultures).
- Determine if the rat is an appropriate model for hematological changes that occur in astronauts during spaceflight.

The results of these hematological studies indicated that on the day of landing (R + 0) there was a significant decrease in the number of Epo-responsive erythroid progenitor cells as enumerated by the BFU-e progenitor cells. Also, the peripheral blood showed a significant decrease in the total WBC and in the absolute number of lymphocytes and monocytes and a slight decrease in eosinophils. Immunophenotyping studies of peripheral blood lymphocytes indicated a significant decrease in the absolute number of B-cells, T-helper cells and T-suppressor cells. All values returned to the control levels by nine days postflight (R + 9). No significant

differences between flight and control animals were observed in the RBC parameters (RBC, Hgb, Hct), serum erythropoietin level, or reticulocyte counts.

The exact mechanism(s) that caused these observed changes during this flight is not completely defined. Although the primary cause might be the influence of microgravity, the etiology is probably multifactorial. Influencing factors might include altered hemodynamics, changes in oxygen demand or oxygen carrying capacity, and metabolic disturbances. In vertebrates, including man and rat, red cell production is controlled by a complex network of hormones and cytokines.

Changes observed in the responsiveness of bone marrow to erythropoietin indicated that differentiation was either slowed or altered in some manner by spaceflight. With progenitor cells always present in the bone marrow, some mechanism(s) is preventing those cells from fully differentiating into mature RBCs. Although Epo was previously considered the major determinant in erythropoiesis, as technology has developed new cytokines are being identified as having an important part in the regulation of erythropoiesis, both positively and negatively. In addition to Epo, those that react positively include Interleukin-3 (IL-3), Interleukin-9 (IL-9), Interleukin-11 (IL-11) and stem cell factor. Negative regulatory factors include tumor necrosis factor-alpha (TNF-a) and transforming growth factor-beta (TGF-b). Since changes were not observed in the Epo levels postflight, it is possible that these new cytokines may be involved, either by increasing, suppressing, or altering the process of erythropoiesis in some form.

As the lymphocyte population decreases, the production of several cytokines could decrease, which could contribute to the reduction in RBC production. As new information becomes available, the enumeration of T-cell subsets, which was the initial step in determining the role of lymphocytes in the complex network of hematopoiesis, may greatly contribute to the understanding of lymphocytes and erythrocytes.

Direct comparisons of data previously published by other investigating teams with data presented in this report is extremely difficult. Differences in the studies include length of flights, strains of rats, postflight animal receiving times, housing (food and water included), and general overall handling of the animals. However, previously reported data are useful in providing a baseline and a guide to the results other investigators acquire. Because the basic scenarios of SLS-2 and SLS-1 were comparable, these data will be corroborated.

#### **Experiment 127:**

# Effects of Zero Gravity on Biochemical and Metabolic Properties of Skeletal Muscle

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On exposure to microgravity, skeletal muscle tissue types show adaptive changes in substrate oxidative capacity as well as transformation of myosin heavy-chain expression in muscles involved in antigravity function and locomotor activity. Muscle tissues from mature male rats on return to Earth from nine days in microgravity show a selective reduction in the capacity of skeletal muscle to process long-chain fatty acids as a fuel to provide energy to support contractile activity. The exact mechanism for this response is presently unclear, but it appears to involve the translocation of fatty acids to the beta oxidative apparatus in the mitochondria. This finding could have an important impact in the endurance capacity of muscle because the capacity to utilize fatty acids is pivotal in reducing the fatigability of the muscle during sustained activity.

On the other hand, in response to microgravity the expression of the two slower myosin heavy-chain isoforms decreases and the two faster myosin heavy-chain isoforms increase in those regions of muscle used extensively for ground-support activity. This fact, coupled with the atrophy that occurs in these types of muscles, reduces the effective muscle mass to support antigravity function and locomotor activity. These findings indicate that spaceflight could impair the normal movement patterns associated with antigravity function and/or postural control in both animals and humans.

## Experiment 141:

## Regulation of Blood Volume during Spaceflight

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This experiment was designed to determine the effect of the microgravity environment of spaceflight on the regulation of blood volume in the rat. This effect was quantified by assessing changes in RBC mass (RBCM), plasma volume (PV), RBC survival, and iron economy. The objective was to evaluate whether the rat was a suitable animal model for further research on the elucidation of the control mechanism responsible for the RBCM loss that has been observed in humans after return from spaceflight.

Radioactive tracers were used to measure PV, RBCM, and RBC survival and iron kinetics. RBCM and PV were measured preflight, (L-8), on landing day, (R+0), and eight days after landing, (R+8). <sup>59</sup>Fe was injected on

R + 0, and its incorporation into RBCs was followed over the next eight days.  $^{51}$ Cr RBC survival studies were made from L - 8 to R + 0 and R + 1 to R + 8.

Upon landing, the mean RBCM of flight rats was significantly less than that of synchronous ground controls, whether expressed as absolute volume or volume normalized for body mass. PV, normalized for body mass, was also significantly lower in the flight animals on R + 0. The <sup>51</sup>Cr survival data do not implicate an increased RBC destruction rate as the cause of the decreased RBCM. The postflight decrease in <sup>59</sup>Fe incorporation into RBCs could indicate a decrease in RBC production in response to either spaceflight or the decreased food intake and weight gain of the flight animals during the postflight period.

Like the human, the rat experiences a decrease in RBCM with microgravity exposure. Neither species shows any indication that this decrease is due to hemolysis. The rat thus appears to be an appropriate model in which to study the mechanisms involved in the control of erythropoiesis during exposure to the microgravity environment of spaceflight.

## Experiment 194: Bone, Calcium, and Spaceflight

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The hypotheses tested in this spaceflight experiment were 1) the type of housing (group vs. individual) will influence the bone response to spaceflight and the recovery from spaceflight; and 2) the response of bone to spaceflight will be localized and will differ not only from bone to bone but also at different sites within the same bone. Growing male rats were flown on SLS-1 on the shuttle Columbia. The rats were housed in groups of five on the shuttle mid-deck or individually in the Spacelab. The flight lasted nine days. Half of the animals were euthanized at the end of the flight period and the other half were allowed to re-adapt to Earth for nine days postflight. The results suggest that housing affects response to spaceflight. The singly housed animals showed greater

in-flight changes and a slower recovery from spaceflight than the group housed rats. These differences occurred in bone mineralization rates, mechanical properties, and enzyme histochemistry. Also, neither all regions of all bones nor all bones were affected by flight; in long bones, the periosteal surfaces showed suppression of formation while endosteal surface showed little change, and no changes were noted in the ribs, calvaria, vertebra, or maxilla, suggesting that the response to spaceflight is not uniform throughout the skeleton.

# **Experiment 238:** Effects of Spaceflight on Gravity Sensors

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Behavioral signs of vestibular perturbation in altered gravity have not been well correlated with structural modifications in neurovestibular centers. The ultrastructural research conducted on gravity sensors (maculas) of rats flown on SLS-1 investigated synaptic plasticity in hair cells of adult utricular maculas exposed to microgravity for nine days. Mammalian maculas are structurally organized for parallel processing of sensory input. There are two types of receptor cells, type I and type II hair cells, and two intrinsic microcircuits comprise the neuronal system. Type I cells communicate only with primary afferent nerve fiber terminals (calyx) that nearly surround them (direct microcircuit). Type II cells lie outside the calyces. They distribute their output to several primary afferents through feed-forward synaptic connections, and they also receive feedback from the afferents. Many synaptic connections are reciprocal (information flows in both directions). Thus type II hair cells are part of the local, or distributed modifying, microcircuit. The hypothesis was that synaptic plasticity would be more evident in type II hair cells because they are modulated by feedback and reciprocal connections to modify macular output.

To test this hypothesis, hair cell synapses, called ribbon synapses, were examined in maculas obtained from flight and control rats after shuttle return (R + 0) and nine days later (R + 9). All rats from SLS-1 were with other investigators and, postflight, were subjected to repeated radioisotope injections and blood withdrawals unrelated to this experiment. Flown rats showed abnormal posture and movement at R + 0 that had essentially disappeared at R + 9. However, the rats at R + 9 had chromodacryorrhea, a sign of acute stress. After conventional preparation of the maculas for ultrastructural study, ribbon synapses were counted in 50 serial sections from medial utricular macular regions of 3 rats of each flight and control group. Counts in 50 additional consecutive sections from 1 sample in each group established method reliability. All

synapses were photographed and located to specific cells on mosaics of entire sections. Pooled data were analyzed statistically. R + 0 maculas had statistically significant increases in total ribbon synapses and in sphere-like ribbons in both kinds of hair cells, whereas in type II cells pairs of synapses nearly doubled and clusters of 3 to 6 synapses had increased twelvefold. All these differences were significant (p < 0.0001). In R + 9 flight animals, synapse counts remained high in both kinds of hair cells. In controls, the number of synapses was elevated in type II cells. Only counts in type I cells showed statistically significant differences at R + 9 (p < 0.0163). High synaptic counts at R + 9 in both flight and control rats may have resulted from stress due to experimental treatments. The results of the SLS-1 experiment nevertheless demonstrate that hair cells ribbon synapses of adult maculas retain the potential for synaptic plasticity, permitting adaptation to the microgravity environment. Type II cells exhibited more synaptic plasticity, but spaceflight induced greater synaptic plasticity in type I cells than had been anticipated.

The results have implications for developmental studies in space and for long-term spaceflight, since the time for recovery of more typical synaptic patterns of distribution of hair cells remains unknown. Some answers should be obtained from the SLS-2 experiment. SLS-2 animals used for gravity-sensor studies were not exposed to extraneous treatments, and tissues were collected in space as well as postflight, permitting comparisons to be made to learn more about the time course of synapse recovery.

#### **Experiment 247:**

#### Changes in Myosin Gene Expression in Fast and Slow Muscles of Rats Exposed to Zero Gravity

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Skeletal muscle fiber types are under neural control, and gravity imposes a constant load on the postural slow muscles. It was postulated that under zero gravity some slow fibers would convert into fast. This possibility was tested in the SLS-1 mission. Juvenile Harlan Sprague Dawley, Inc., rats were flown in the Spacelab for nine days, and their slow soleus and fast extensor digitorum longus (EDL) muscles were harvested upon return to Earth nine days later. Control rats corresponding in age to the launch, flight, and postflight animals were also studied. Muscles were analyzed immunohistochemically using monoclonal antibodies (MAbs) against myosin heavy chains (MHC). The results showed that a considerable proportion of control soleus fibers expressed both fast and slow MHCs, and fibers expressing fast MHC decreased with age. MAb 5-4D, specific for slow (type I) MHC, stained 84.1  $\pm$  2.2 percent (s.e.) of flight soleus and

 $87.6 \pm 3.4$  percent of age-matched control soleus fibers. MAb 1A10, specific for all fast (type II) MHC isoforms, stained  $45.7 \pm 1.5$  percent of flight soleus and  $26.9 \pm 2.1$  percent of control soleus fibers. Thus muscles of flight rats showed a marked increase in proportion of fibers expressing fast MHC. Using MAb5-2B specific for IIA/IIX but with a lower affinity than 1A10, a somewhat smaller increase in fast-MHC binding fiber proportion was detected in soleus of flight rats. These changes were sustained at nine days postflight. Slow soleus fibers decreased in diameter 26.5 percent while fast soleus fibers decreased by 20.2 percent, relative to age-matched controls. Changes in fiber type distribution were not detected in the EDL. However, EDL slow and IIA fibers showed small but significant increases in fiber diameter. The results show that the effects of zero gravity are muscle specific, and they confirm the postulated shift in myosin gene expression from slow to fast in antigravity muscles, but fiber type change is incomplete within nine days.

#### **Experiment 303:**

#### Effects of Microgravity on the Electron Microscopy, Histochemistry, and Protease Activities of Rat Hindlimb Muscle

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The process of skeletal-muscle deterioration induced by spaceflight was studied in rats exposed to microgravity for nine days aboard SLS-1. On R + 0, 15 flight rats were euthanized, approximately one every 15 minutes, permitting an unprecedented analysis of the temporal effects of short-term reexposure to gravity. To account for the effects of the flight caging environment and other experimental treatments, a DFPT using a replicate set of animals was conducted in the flight cages at 1 g to otherwise simulate the mission. Spaceflight induced significant atrophy (≠ 40 percent) of adductor longus and soleus muscle fibers and increased expression of fast myosins (mainly types IIA and IID/X, some IIB), which resulted in about half of slow type I fibers coexpressing slow and fast myosin. Since alteration of myosin content is relatively slow, expression of fast isoforms most likely represents an in-flight change. The respiratory diaphragm, a nonantigravity muscle, showed 19-percent atrophy; this unexpected finding suggests that the weight of viscera on Earth impacts diaphragm-muscle fiber size. The atrophic adductor longus muscle of the flight rats showed increasing pathological damage over the 2- to 7-hour postflight period of resumption of weight-bearing activity. Damage included thrombosis of the microcirculation, interstitial and cellular edema, muscle-fiber fragmentation, sarcomere disruptions, activation of phagocytic cells, and

elevated ubiquitin conjugation. Edema was present at the earliest time point examined (2 hours), whereas sarcomere eccentric-like lesions did not occur until about 4 hours postflight. Compared to the L + 0 vivarium controls, involution of neuromuscular junctions was significantly more prominent in both the flight and the other control rats, indicating nonspaceflight effect. A partial explanation was a vitamin B1 (thiamine) deficiency in the Teklad space food bars. The soleus showed much fewer pathological symptoms than the adductor longus. Inspection of the videotapes of rat behavior on R + 0 indicated a greater resumption of loaded contractile activity by the adductor longus relative to the soleus. These studies on rats point out the need to develop protocols for humans to insure safe transition from microgravity to terrestrial gravity following long-term spaceflight.

#### Effects of Weightlessness on Aurelia Ephyra Differentiation

Dear Colleague Letter Dorothy Spangenberg Eastern Virginia Medical School, Norfolk, Va.

The Aurelia metamorphosis test system was used to determine space environment effects on the development and behavior of tiny ephyrae. Polyps, which were induced to produce ephyrae following iodine or thyroxine treatment, and Earth-developed ephyrae were flown. The purpose of the experiment was to determine whether metamorphosis of polyps into ephyrae could proceed in space, and if so, to determine whether ephyra graviceptor development (including statolith formation) and swimming/pulsing behavior inflight were normal as compared with ground controls.

#### **Results:**

Numerous ephyrae formed in space during the nine-day SLS-1 mission. These animals were essentially normal in morphology and in the number of statoliths formed in their graviceptors. In Earth-formed ephyrae sent into space, however, statolith numbers were significantly reduced (as compared with controls) while they were in space, indicating that demineralization of the calciumcontaining gypsum was enhanced.

Also while in space, both ephyrae from Earth and those that developed in space tended to swim in circles and could not orient when they stopped swimming. Apparently, important positional cues from the graviceptors to the neuromuscular system were affected. Upon return to Earth, swimming ephyrae oriented according to the g vector, but six times more space-developed ephyrae had pulsing abnormalities than controls. This finding indicates that either the neuromuscular structures of these animals did not form normally while in space or the animals were

unable to adapt to the 1-g environment upon return to Earth.

The metamorphosis process in the jellyfish is influenced by a hormone (jellyfish thyroxine), which is synthesized following iodine administration. Two groups of jellyfish polyps in space, however, formed ephyrae without iodine administration, indicating that hormone synthesis, utilization, or excretion was different in space-exposed animals. Jellyfish thyroxine differences may also be linked to the increased statolith demineralization and normal pulsing found in ephyrae from space.

#### 5.3 Biospecimen Sharing Program (BSP)

The BSP was developed to insure maximum utilization of tissues from the limited number of rats flown on SLS-1. The initial tissue sharing program was limited to the PIs selected for flight experiments from the Announcement of Opportunity in 1978 (AO-78). As the SLS-1 payload was defined, it became evident that valuable tissue samples not needed by these investigators could be made available to the scientific community and thus maximize the scientific return from the mission. Acceptance for participation in the SLS-1 BSP was initially limited to an extension of the joint United States/Union of Soviet Socialist Republics (U.S./USSR) studies on the USSR Cosmos flights investigating metabolic, structural, and functional changes in the rat body under the influence of a shortterm exposure to microgravity. After the incorporation of the USSR experiments, other studies were accepted from France, Germany, Canada, and U.S. government and university laboratories. The total experiment complement was 31 experiments comprised of 7 AO-78 PIs, 17 primary U.S./USSR BSP studies, and 1 Canadian, 2 French, 1 German, and 3 U.S. secondary experiments.

# Results of U.S./USSR Joint Biospecimen Sharing Program

Translated by Galina Tverskaya Lockheed Martin Missiles & Space, Moffett Field, Calif.

## **Experiment SLS-1-01 Bone Biomechanics**

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After nine days of flight and nine days of recovery mechanical properties and mineral content of spongy bone exposed to multiple cyclic compressions were investigated. No significant changes in mineral content were observed; the variations indicated only a trend toward diminished mineralization when compared to the

age-matched controls. However, mechanical properties of rat bone significantly deteriorated. Preliminary analysis of the cyclic-compression results revealed significant differences in bone behavior nine days after recovery. These changes can be attributed to an increase in the number of poorly mineralized juvenile structures in rat bones.

#### **Experiment SLS-1-02**

#### Metabolic and Structural Changes in Bone and Systems Regulating Bone Growth and Metabolism

A. S. Kaplansky and I. A. Popova Institute of Biomedical Problems, Moscow, Russia

E. Morey-Holton

NASA Ames Research Center, Moffett Field, Calif.

#### I. Bone Histomorphometry

G. N. Durnova and E. I. Ilyina-Kakueva Institute of Biomedical Problems, Moscow, Russia

#### II. Bone and Plasma Biochemistry

I. A. Popova and N. Yu. Fedotova Institute of Biomedical Problems, Moscow, Russia

#### **III. Bone Elemental Composition**

T. E. Burkovskaya

Institute of Biomedical Problems, Moscow, Russia

V. M. Nazarov, M. V. Frontasyeva, and

S. F. Gundorina

Joint Institute for Nuclear Research, Dubna, Russia

# IV. Histology and Immunocytochemistry of Thyroid Glands

V. I. Loginov

Institute of Biomedical Problems, Moscow, Russia

# V. Histomorphometry of Pituitary Somato-tropic Cells

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Morphological and biochemical examinations of bone, plasma, and endocrine systems involved in the regulation of bone metabolism were performed using rats flown for nine days on the U.S. biomedical laboratory SLS-1. Histomorphometric study showed that nine days of weightlessness caused early, poorly expressed signs of osteoporosis of the spongiosa of tibial proximal

metaphyses, viz., a decrease of bone volume in the secondary spongiosa and an increase of the bone resorption surface. In the spongiosa of lumbar vertebrae and cortical bone of the tibial diaphyses no signs of osteoporosis were detected. These changes in tibial metaphyses correlated with biochemical variations, which included a trend toward a decline in alkaline phosphatase (an enzyme involved in bone formation) and a trend toward an increase in tartrate-resistant acid phosphatase (an enzyme involved in bone resorption). Neutronactivation analysis of the bone elemental composition showed that exposure to weightlessness was followed by a reduction of calcium, phosphorus, sodium, and chlorine, which was in good agreement with the inhibition of thyroid C-cells producing calcitonin required for normal mineralization of bone matrix. An increased concentration of calcium and a decreased concentration of phosphorus in blood indicated that mineral-balance changes occurred in the mammalian body at early stages of adaptation to weightlessness. The pituitary glands of weightless rats showed an inhibition of the functional activity of somatotropic cells (decline of the synthesis and secretion of growth hormone). This finding was consistent with findings of previous Cosmos experiments. Nine days after recovery most parameters under study returned to the norm. On the whole, the changes seen in bone and endocrine organs involved in bone metabolism regulation were similar to those observed after longer term spaceflights; in quantitative terms, they were less pronounced than in the rats flown for seven days on Cosmos 1667. This difference can be attributed to the difference in the rat strains flown on SLS-1 and Cosmos 1667 and to the difference in the experimental designs.

#### **Experiment SLS-1-03**

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The effect of a nine-day spaceflight and a nine-day recovery period on bone osteogenesis was investigated using induction of ectopic osteogenesis by a demineralized matrix of femurs of flight rats (donors). Preliminary analysis of the results has shown that in space osteo-inductive activity of bone matrix increased but remained qualitatively unaltered. The amount of de novo generated bone was not large in recipient rats (less than in the controls), but the level of its mineralization was significant. During the nine days after flight, osteo-inductive potentials of the matrix decreased and inhibitory activity increased; in

other words, bone regenerative potentials declined, thus stimulating osteoporosis.

# Experiment SLS-1-04 Lipid Peroxidation and Antioxidant Protection System

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In order to study the effect of weightlessness and other spaceflight factors on rat lipid peroxidation (LPO) and antioxidant protection (AOP), the following parameters were measured in the homogenates of the liver, kidneys, brain, skeletal muscles, myocardium, and plasma: LPO products—diene conjugates, malonic dialdehyde, Schiff's bases, tocopherol, and major lipid antioxidant. In addition, total antioxidative activity was determined in plasma, and antioxidative enzymes (superoxide dimutase, catalase, glutathione peroxidase, and glutathione reductase) were determined in tissues.

The experiments gave evidence that the nine-day flight on SLS-1 did not produce a significant effect on the LPO intensity or AOP system. Changes in the LPO and AOP parameters were found only nine days after flight. They were, evidently, associated with a high workload the organs had to perform during re-adaptation to the Earth's gravity. The fact that the plasma parameters remained unchanged indicated that free radical processes in the animals were compensated for after flight.

#### Experiment SLS-1-04A Erythrocyte Metabolism and Membrane State

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The present investigation demonstrated changes in cellular metabolism, probably caused by alterations in the structure and function of cellular membranes. Those alterations included changes in phospholipids and an enhanced rate of the Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>++</sup>-pump. These membrane changes were required to preserve cell integrity. The changes were adaptive in nature, because nine days after recovery the membranes returned to the norm.

#### **Experiment SLS-1-05**

# Mechanism of Development of the Hypersecretory Syndrome of the Stomach

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Exposure to spaceflight causes significant changes in the morphology and function of the digestive system, the most significant being the development of the hypersecretory gastric syndrome. Study of the stomachs of rats after a nine-day spaceflight revealed an increase in the peptic potential of the stomach, which was at its highest at day R + 9. The hypersecretory gastric syndrome is characterized by an increase in the activity of the chief cells of the stomach that produce pepsinogen and an increase in hydrochloric acid in the stomach between digestion events. An increase in the acidic-peptic potential correlated with an increase in gastrin, which is the primary physiological activator of the parietal cells of the stomach. These changes taken together facilitate enhanced aggression of gastric juice toward the mucous membrane and stimulate ulceration.

#### Experiment SLS-1-06 Mechanism of Changes in the Exocrine Function of the Pancreas

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Study of pancreatic function after a nine-day spaceflight revealed changes in the activity of digestive enzymes. At day R+9, amylolytic activity of the pancreas increased significantly. Spaceflight produced no significant effect on trypsinogen. At day R+9, lipase activity decreased substantially. The development of pancreatic insufficiency in response to spaceflight requires further detailed study.

The function of the gastrointestinal tract is characterized by the continuity of hydrolytic degradation of nutrients. Interaction of the stomach, pancreas, and small intestine in the course of re-adaptation to the Earth's gravity is an example of the self-regulatory function of the gastrointestinal system in controlling enzyme activities.

#### **Experiment SLS-1-07**

# Study of the Digestion-Transport Conveyor in the Small Intestine

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Study of the small intestine after a nine-day spaceflight revealed various changes in enzyme activities. With respect to protein hydrolysis in the membrane, dipeptidase activity changed in the proximal and distal segments in a different way, suggesting a compensatory nature of the changes. Lipid changes included a lower activity of monoglyceride lipase and a higher activity of alkaline phosphatase in the proximal segment of the small intestine, which also pointed to compensatory-adaptive changes. Carbohydrases remained essentially unchanged.

Changes in the digestion-transport hydrolysis of proteins, fats, and carbohydrates were functional and reversible. The adaptive pattern of changes in membrane digestion was indicated by the self-regulatory activity of the digestive system in relation to the distribution of enzyme activities.

#### **Experiment SLS-1-08**

# **Effect of Spaceflight Factors on the Functional Activity of Immune Cells**

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The goal of the experiment was to study the effect of a nine-day spaceflight on the function of immuno-competent cells of rats (basal control, R+0, and C+0 rats) and to follow the dynamics of the rats' recoveries after return to the Earth's gravity (R+9 and

C + 9 rats). Also, an additional tail-suspension experiment was performed (S + 0 and CS + 0 rats).

Proliferative activity of spleen T-lymphocytes was measured in 48-, 72-, and 96-hour tests with concanavallin A (0.1, 1.0, and 10.0 mg/ml) and interleukin-2 (2 U/ml) with respect to the incorporation rate of <sup>14</sup>C-uridine and <sup>3</sup>H-thymidine. When compared to the corresponding controls, T-cell activity remained unchanged in the R + 0 rats, increased in the R + 9 rats (in the cultures containing no mitogen, containing the mitogen in the cultures in optimal and high concentrations, and in the cultures simulated by IL-2), and decreased in the S + 0 rats (in the cultures containing no mitogen, in the cultures containing low concentrations of concanavallin A, and in the cultures incubated for short times).

Natural cytotoxicity of spleen and bone-marrow cells in the membranotoxic test with target cells YAC 1 was increased in the R+0, R+9, and S+0 rats (in the latter case only in spleen cells). Activity of spleen natural killers toward target cells K562 was also increased (the increase was insignificant in the S+0 rats) and that of bone marrow was decreased (in the R+0 rats the decrease was insignificant and in the R+9 and S+0 rats it was more noticeable).

The capability of spleen-cell cultures to synthesize humoral mediators of immunity was investigated. The production of interferon-alpha remained essentially unaltered. The rate of interferon-gamma synthesis decreased neither after flight nor after suspension. Activity of IL-2 in cellular supernatants did not change in the R + 0 and R + 9 rats, but decreased in the S + 0 rats. Activity of TNF-alpha in the supernatants of rat spleen cells was increased after flight (R + 0 rats) while that of TNF-beta did not change. The production of TNF-beta declined in the S + 0 rats.

#### **Experiment SLS-1-09**

## **Brain Primary Perceptive Structures: Their Morphology and Histochemistry**

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The results of electron microscopic examinations of the cortex of the medial and lateral nodulus of the cerebellum of rats flown for nine days on SLS-1 revealed ultrastructural changes in nerve and glial cells of the granular and molecular layers. These changes reflect the functional changes that occurred in orbital flight and after recovery. Ultrastructural changes in some glomerules, granular cells, and glia of the granular layer, as well as in axonal

terminals, axo-spine contacts, axo-dendrite synapses. dendrites, and glia of the molecular layer indicated that in microgravity both vestibular input to the nodulus cortex and vestibular afferent input to the Purkinje cells decreased. At the same time, electron-microscopic changes of other structural elements of nerve cells in the granular and molecular layers pointed to excitation (or overexcitation and morphological signs of synaptic transmission blockade) of vestibular input structures. The excitation in turn reflected enhancement of vestibular afferent signals that reached the nodulus from two to three hours after recovery, probably because of enhanced sensitivity of the otolith apparatus in microgravity. In contrast to structural elements of vestibular input to Purkinje cells, no ultrastructural changes were seen in axo-dendrite synapses formed by climbing fibers transmitting visual impulses, which were located on the Purkinje cell dendrites. This observation showed that visual impulses to Purkinje cells in microgravity remained unaltered. After nine days of re-adaptation to the Earth's gravity, ultrastructural signs of a reduced flow of vestibular signals were virtually absent; however, morphological evidence of excitation was detectable in some granular cells and in axo-spine contacts of the molecular layers. The flight rats showed predominantly Purkinje cells of dark type. However, the data available about correlations of ultrastructural and functional changes in Purkinje cells are insufficient to make conclusions concerning their functional state in microgravity.

#### Experiment SLS-1-10 Morphology of Neurons

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Morphometric investigation of the dendrite geometry in giant multipolar neurons of nucleus reticularis gigantocellularis in the medulla oblongata of rats flown for nine days on SLS-1 did not reveal significant differences between flown and ground control animals. However, significant differences in the number and mean branching of dendrites between R + 0 rats and R + 9 rats suggested rearrangement of the dendrite tree of neurons that developed during and after flight. Comparison of those findings with the data obtained during similar studies in Cosmos 1667, Cosmos 1887, and Cosmos 2044 flights helped identify time-course variations of the dendrite tree of

gigantic multipolar neurons of the reticular formation at different stages of animal adaptation to microgravity.

# **Experiment SLS-1-11 Cerebral Cortex Ultrastructure**

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Electron-microscopic examinations of the motor, somatosensory, visual, and olfactory cortex of rats flown for nine days on SLS-1 demonstrated ultrastructural changes in neuronal and glial cells. These findings suggest that functional changes of the cortical structures occurred both during and after flight. In the somatosensory and motor cortex, ultrastructural changes pointed to the following developments in microgravity: 1) drastic decrease of afferent flow to the cortex; and 2) reduction of the afferent flow to large pyramidal neurons in the V layer. This flow reduction is evidently responsible for the hypofunction of spinal motoneurons, which was previously detected morphologically by other authors. At the same time, the ultrastructure of some axonal terminals, axo-dendrite synapses, and stellate cells indicated that the synapses and stellate cells were in the excitation state, which was associated with an increased afferent flow to the cortex during the first two to three hours after recovery. The ultrastructure of the somatosensory and motor cortex nine days after recovery indicated both an enhanced afferent flow to the cortex on Earth and an increased functional activity of large, pyramidal neurons of the V layer. In the visual cortex of flight rats, ultrastructural changes were similar to those of the somatosensory cortex but less significant; also evidenced was a slight decrease of the afferent flow to the visual cortex in microgravity. At the same time, the high functional activity of synapses of the IV layer of the visual cortex and that of neurons suggested that the visual flow increased after recovery. However, nine-day exposure of the animals to the Earth's gravity normalized the functional state of structural elements of the visual cortex. Ultrastructural changes of the olfactory cortex suggested a slight decrease of the afferent flow and an increase of the functional activity postflight. The ultrastructure of axonal terminals, dendrites, synaptic contacts, and postsynaptic structures, the increase in number of axonal and dendrite growth cones, the enlarged area occupied by glial cell processes, and a greater number of capillaries in the cortical structures of the rats flown for nine days pointed to an active restructuring in the cortical connections, which formed the structural foundation for the adaptation of the cerebral cortex to microgravity.

# **Experiment SLS-1-12 Cytochemistry of Neurons**

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Results of cytophotometric and cytochemical examinations of acetyl cholinesterase (ACE), monaminoxidase (MAO) and glutamate dehydrogenase activity in the III and V layers of the somatosensory cortex and the head of the caudate nucleus of the brains of rats flown for nine days on SLS-1 indicate that the exposure diminished MAO in fibrillar structures of the V layer of the somatosensory cortex and the head of the caudate nucleus and reduced ACE in neuronal bodies of the head of the caudate nucleus. These changes can be interpreted as an indication of a decline of 1) the modulating effect of monaminergic structures on the somatosensory cortex and the head of the caudate nucleus and 2) the inhibitory effect of neurons of the caudate nucleus on globus pallidus, n. ruber, substantia nigra, and other cerebral structures.

# **Experiment SLS-1-13 Contractility Properties of Skeletal Muscles**

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The effects of zero gravity on contractile properties of skeletal muscles of rats were studied after a nine-day spaceflight and nine-day postflight re-adaptation period. The results indicated that the greatest changes occurred in the postural soleus muscle: the diameter of muscle fibers diminished, and isometric tension and contraction velocity decreased. The fast locomotor muscles, i.e., EDL and both heads of the gastrocnemius muscle, showed a trend toward an increase in the contractile force. EDL also showed a decrease of the contraction and half-relaxation velocity. During the recovery period these parameters returned to the baseline. These observations indicate that

changes in contractile properties of muscles during flight largely depend on their functional profile.

# Experiment SLS-1-14 Weightlessness Effect on Water and Electrolytes in the Animal Body

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After a nine-day SLS-1 flight the content of water, sodium, potassium, calcium, and magnesium was measured in the livers, kidneys, hearts, skin, skeletal muscles, and bones of male rats. On the day of recovery the content of water, sodium, and potassium diminished in the heart. In other tissues no changes were seen.

## Experiment SLS-1-15 Spinal Cord: Morphology and Histochemistry

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Quantitative cytochemical and histochemical analysis was made of acetyl cholinesterase, cytochrome oxidase, and alkaline phosphatase activity in the anterior horns of the cervical and lumbar enlargements of spinal cords of rats flown for nine days on SLS-1 and then exposed to Earth's gravity for two to three hours or nine days. Results indicate that the activity of those enzymes remained unchanged at the C2–C4 level and that the activity of cytochrome oxidase decreased at the L1–L3 level. The latter finding suggests that, as early as nine days after exposure to weightlessness, the function of the motoneurons in the lumbar enlargement diminished. However, the fact that nine days after recovery

cytochrome oxidase activity in motoneurons of the lumbar enlargement returned to the norm indicates that the changes were reversible and that functional activity of motoneurons was easily restored. Increased numbers of "active capillaries" in the anterior horns of the lumbar enlargement of the spinal cord at day R + 9 points to enhanced transport of metabolites across capillaries and suggests the development of compensatory processes that stimulated metabolism in the spinal cord after recovery.

## Experiment SLS-1-16 Histochemistry of the Hypothalamus

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Using quantitative histochemical methods, the activity of glutaminase, the key enzyme of glutamate synthesis in the nervous tissue, as well as the content of lipids and defatted dry substance was measured in single fragments of freeze-dried sections (20 microns thick) of the arcuate nucleus (AN) and medial eminence (ME) of the hypothalamus of rats flown for nine days on SLS-1. The weight of single AN and ME fragments dissected from freezedried sections was 0.2 to 1.0 mg. It was found that glutaminase activity in AN decreased by 22.7 percent and in ME by 30.4 percent, while the proportion of lipids and defatted dry tissue remained unaltered. Reported data about the high sensitivity of somatoliberin-containing neurons of the AN to glutamate and the present findings suggest that glutamate may be involved in the regulation of growth-hormone excretion.

# **Experiment SLS-1-17 Morphology of Neurons of the Cerebral Cortex**

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A morphometric examination of the geometry and orientation of dendrites of pyramidal neurons of the III layer of the visual cortex of rats flown for nine days on SLS-1 has been completed. The resultant data have been processed using the Q factor analysis, discriminant analysis, and

K-means splitting method. The findings have shown an increase in the length of apical dendrites located in the upper layers of the visual cortex. These dendrites are part of pyramidal neurons of the III layer, which have well-developed apical systems and participate in the establishment of associative connections between various cortical compartments. This process, which can be induced by the need of an additional afferent input, acts as a foundation for new connections between the visual cortex and other cortical compartments in microgravity. An enlargement of the profile size of the body of pyramidal neurons of the III layer, also observed after flight, can be viewed as another indication of the restructuring of the dendrite system of these neurons in microgravity.

# ANF-Sensitive Guanylyl Cyclase Activity in Rat Liver Tissues Flown on SLS-1

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Atrial natriuretic factor (ANF) secretion has been shown to increase if there is a cephalid shift of body fluids, and to decrease if there is long term elevated plasma concentration. Among the receptors, the membrane-bound guanylyl cyclase appears to mediate most of the effects of ANF. In weightlessness, with the known redistribution of body fluids toward the head, it was postulated that the secretion of ANF may be enhanced and the consequent reduction of receptors may occur. To determine the effects of microgravity on the responsiveness of the ANFguanylyl cyclase system, analyses of liver tissues from 10 rats flown on SLS-1 for nine days were made. The results were compared to data from tissues from control animals not exposed to spaceflight. Control tissues came from 10 rats sacrificed at the time of launch and a flight control group of 10 maintained on the ground in flight equipment exposed to the environment experienced by the flight rats with the exception of the stresses of lift-off, microgravity, and recovery. Guanylyl cyclase activity was measured in the liver-membrane fraction, and enzyme activity was stimulated by the following ANF analogs: ANF-(99-126) (ANF), ANF-(95-126) (urodilatin), ANF-(103-123) (AP 1) and C-type natriuretic peptide (CNF). Formed cyclic guanosine monophosphate (GMP) was measured by radioimmunassay. All analyses were done in blinded fashion. In all samples analyzed, the ANFstimulated guanylyl cyclase activity was about twofold. The samples from the flight and control groups were almost identical. In contrast, samples from launch control samples were twofold higher than the "flight" groups. Stimulation with the various analogs showed the same response pattern for all three groups. These identical patterns indicate that there is no apparent altered receptor subtype distribution during weightlessness. This project also showed that:

1) measurement of ANF-sensitive guanylyl cyclase activity has been successfully performed in a multinational BSP; 2) the inclusion of appropriate groups has contributed considerably to the proper evaluation of in-flight samples; and 3) the activity of the guanylyl cyclase is unaltered in tissues exposed to microgravity. Conclusions of this study indicate that the cellular response to circulating ANF is unaltered during spaceflight.

#### Norepinephrine Content in Discrete Brain Areas and Neurohypophseal Vasopressin in Rats after a Nine-Day Spaceflight

#### ANP Binding Sites in Choroid Plexus of SLS-1 Rats

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Two studies, concerning norepinephrine (NE) and vasopressin (AVP) contents (ref. 16) and atrial natriuretic peptide (ANP) binding sites (ref. 17) were performed on brains and neurohypophysis obtained from SLS-1 rats, which were orbited for a nine-day spaceflight and compared with eight control groups.

The NE content was significantly decreased in the locus coeruleus of flight rats  $(2.9\pm0.3~{\rm vs.~}8.9\pm0.7)$  pmol.structure<sup>-1</sup>, p < 0.001), and was restored at the control level after a nine-day recovery period. The NE content remained unchanged in A2 and A5 brainstem nuclei. The AVP content was increased in the posterior pituitary of flight animals  $(1.47\pm0.1~{\rm vs.~}0.86\pm0.1~{\rm g}$  structure<sup>-1</sup>, p < 0.01) and was significantly decreased in their hypothalamus  $(8.95\pm2.20~{\rm vs.~}2.2~{\rm g}$  structure<sup>-1</sup>, p < 0.05). The authors conclude that the NE depletion in locus coeruleus and the alteration of AVP release were consistent with an acute stress occurring during and/or after landing, which tended to mask the neuroendocrine modifications caused by microgravity.

In parallel, ANP binding sites were analyzed by autoradiography. Computer-assisted micro-densitometric image analysis was used, in choroid plexus and meningia of the same rats flown for nine days on the mission STS-40. ANP binding sites were significantly increased in choroid

plexus of lateral and third ventricles of flight rats compared with control rats  $(413 \pm 43 \text{ vs. } 163 \pm 69 \text{ fmol.mg})$ prot. -1, p < 0.01 and  $457 \pm 14$  vs.  $292 \pm 47$  fmol.mg prot. -1, < 0.05, respectively). No significant differences in binding affinity were observed at the level of these structures. Choroid plexus from the fourth ventricle displayed changes in neither binding capacity nor affinity after spaceflight. Meningia from the flight rats demonstrated no significant modifications in the number of ANP binding sites, but displayed a significant increase in K<sub>d</sub> values  $(0.462 \pm 0.062 \text{ vs. } 0.102 \pm 0.045 \times$  $10^{-9} \text{ M}^{-1}$ , p < 0.01). This finding suggested a reduced affinity of the meningeal ANP receptors after a nine-day spaceflight. The authors conclude that atrial natriuretic peptide might be involved in the regulation of fluid and electrolyte fluxes in the brain during adaptation to microgravity. The modification is evidenced in the expression of specific, high-affinity receptors, mainly choroid plexus from forebrain or in meningia.

#### Effect of Microgravity on the Relations Between Microbiological and Epithelial Tissue and Functions of the Gastrointestinal Tract

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Unite d'Histopathologie Institut Pasteur, Paris, France (no final report)

#### Atrial Natriuretic Factor (ANF) Changes in the Heart

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(no final report)

# Effect of Spaceflight on Cardiac Enzyme Activities Involved in Energy Metabolism

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(no final report; investigator had left the NHLBI before the tissues were delivered)

#### **Histologic Examination of Lung Tissue**

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(no final report)

# Growth Hormone Releasing Factor (GRF) Binding Sites of Pituitaries Obtained from Spaceflight

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Growth-hormone secretion is compromised during exposure to actual or simulated (rat hindlimb suspension) microgravity. Further, growth-hormone secretions in response to GRF are markedly reduced in pituitaries of suspended rats. These data suggest that GRF receptors may be reduced by microgravity, in number or binding affinity. Thus this study was designed to determine whether or not the GRF binding sites are altered in terms of their number (binding capacity) or affinity upon exposure to microgravity. Radiolabeled GRF (human) was used as a radioligand and, with rat GRF as a cold competitor, the receptor assay was performed on the pituitary homogenates prepared from male albino rats flown on the SLS-1. No specific (receptor) binding was found, presumably because of the severe desiccation of the glands. In contrast, homogenates prepared from freshly dissected glands, using the same procedure, provided a doseresponse curve.

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### APPENDIX 1

ARC Space Life Sciences Payloads Office Overview

### AMES RESEARCH CENTER OVERVIEW

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Received December 4, 1991

SPACELAB LIFE SCIENCES 1
(SLS-1) AMES RESEARCH CENTER HARDWARE

SPACELAB LIFE SCIENCES 1
AMES RESEARCH CENTER TRAINING

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Received December 19, 1991

ARC SPACE LIFE SCIENCES ONE (SLS-1)
BASELINE DATA COLLECTION

ARC SPACE LIFE SCIENCES ONE (SLS-1)
BIOSPECIMEN SHARING PROGRAM

# Spacelab Life Sciences 1 (SLS-1) Ames Research Center Hardware

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Received December 4, 1991

#### **BACKGROUND 1978 TO 1981**

ARDWARE FOR THE Ames Research Center (ARC) experiments aboard Spacelab Life Sciences 1 (SLS-1) started with concepts for animal holding facilities for rodents, squirrel monkeys and rhesus monkeys and a general purpose work station as part of the Spacelab Mission Development test #3 (SMD-3) conducted at the Johnson Space Center (JSC) in 1977.

The current Research Animal Holding Facility (RAHF) and General Purpose Work Station (GPWS) were originally designed and built in the 1978/1981 time-period for flight on Spacelab 4 (the term originally applied to SLS-1) which was scheduled for a 1981 launch as the first dedicated Life Sciences mission. In the interim, RAHFs were flown as an "Engineering Proof of Concept" aboard Spacelab 3 (SL-3) in April/May 1985.

Two versions of RAHF were built, one to house 24 rodents and one to house four unrestrained squirrel monkeys. The hardware was built at Lockheed Missiles and Space Company (LMSC) and delivered to the Space Life Sciences Payloads Office (SLSPO, then the Life Sciences Flight Experiments Project) in 1982. The General Purpose Work Station (GPWS) was developed in the same time frame but due to budget cuts and launch slips, the hardware was not delivered to the project until 1984.

#### Research Animal Holding Facility (RAHF)

The RAHF was designed to provide basic animal maintenance of air, food, water, waste management, lighting, humidity removal, and temperature control. Water was available to the animal in each cage compartment via a set of lixits mounted just above the cage top in the the cage module. Food was dispensed via a feeder cassette mounted on the side of the cage which required changeout by the crew every three days. Waste management was controlled through the use of airflow to direct urine and feces into a waste tray at the bottom of the cage. Temperature and excess humidity removal were controlled via an Environmental Control System (ECS) mounted on the rear of the cage module. A water separator system removed excess humidity and transferred this liquid to a condensate collector bag. The bag was changed at a 'quik disconnect' fitting, as required, by the crew. Lighting was incorporated into the cage module with the lights mounted just above the cage tops. Activity of the rodent was monitored via an infrared beam activity monitor. Figure 1 illustrates

these elements of the early RAHFs. A camera structure was mounted over a four-cage segment on the rodent RAHF and was activated during launch and reentry on Spacelab 3.

During the SL-3 flight, problems were encountered with the hardware; chief among these was particulate contamination and animal odor. Particulates observed by the crew and collected in fan filter screens in the spacelab module included food bar crumbs, fine charcoal bits, and fecal particles, which were released from the cage during feeder and waste tray changeout. Persistent animal odor was also reported by the crew. Following the SL-3 flight, at the direction of NASA's Associate Administrator for the Office of Space Science and Applications (OSSA), Bert Edelson, a committee, chaired by Harley L. Stutsman of JSC, was convened to review the design of the RAHF and recommend changes. Thirty-one review item discrepancies were noted with the design.

Extensive post-flight testing of the RAHF hardware revealed several leak paths within the cage module which prevented operation of the unit as a negative pressure device. The outward direction of the air leaks accounted for the presence of odor in the cabin. The rodent cages were constructed without adequate sealing, e.g., the cage top was 1/4" grid, two holes in cage top for lixit access, waste trays not sealed at cage front, severely crumbing foodbar, etc. Airflow was also demonstrated to be highly erratic, turbulent within the cage, and non-existent in some places.

As a result of the SL-3 problems, the RAHF was eliminated from the SLS-1 payload and in its place, the Ames experimenters proposed flying Animal Enclosure Modules, to preserve the capability for evaluating the effectivity of microgravity on rats, as an experimental model. Because of the time element, this was the only means of having the non-human experiments represented in the dedicated Life Sciences Spacelab proposed as a 1986 launch, at that time.

Between 1985-1988, the RAHF was redesigned to prevent the recurrence of the particulate and odor problems. New versions of the RAHF were delivered to the SLSPO in August 1988 and June, 1989. Due to the launch delay to 1990, the RAHF was remanifest on SLS-1 in July, 1987, following the CDR and unanimous acceptance of the new design by the Crew and Oversight Committee.

To assure requirements compliance with all elements in the redesign of the RAHF, a Requirements Document was developed and signed by the Principal Investigators, the Astronaut Office at JSC, the Mission Management Office for

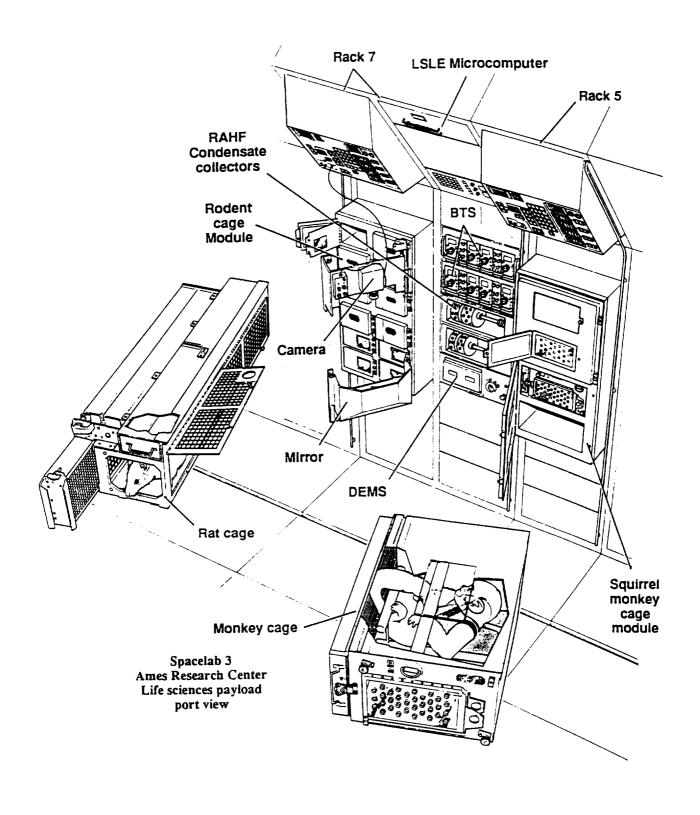


FIGURE 1. SL-3 RAHF Configurations

SLS-1, and the Life Sciences Division at NASA Headquarters. Hardware changes in the specification forwarded to LMSC included:

- Sealing the cage module to prevent odor escape and to insure inward airflow.
- Improving the ECS system to produce linear airflow through the cages.
- Redesigning the cage to include internal lixits, an improved waste tray, and feeder with expanded food capacity.
- Assuring that all cage parts including feeder, waste, tray, and cage are totally interchangeable (proven during SLS-1 flight integration).
- Sealing the cages to prevent escape of all particles >150 microns.

Modifications were implemented to facilitate various RAHF problems observed:

- Added Single Pass Auxiliary Fan (SPAF) to produce high inward airflow during cage servicing operations such as feeder or waste tray replacement.
- Replaced all drinking water system parts with stainless steel. The previous system had been susceptible to corrosion.
- Added iodinator system to reduce drinking water contamination.
- Implemented reliability upgrades as required in the water separator fan and other critical components.
- Sealed cages to cage module to prevent escape of particles into the cabin. All exhaust air to the cabin to be filtered to 0.3 microns via use of HEPA filters.
- Addressed and corrected all Problem Reports generated at the Kennedy Space Center during the previous SL-3 integration activities.

The Astronaut Office at JSC was asked to participate in the redesign activity as they were the eventual hardware users. The SLS-1 crew including Rhea Seddon, Jim Bagian, Bob Phillips, Drew Gaffney, and Millie Hughes-Fullford, were extremely helpful in the design, e.g., cage latches, SPAF configuration, waste tray design, rodent viewing window.

As a method of determining the RAHF airflow problems on SL-3 and altering them, an existing oil pipeline design software program was modified to simulate the airflow in the RAHF. The program allowed analyses of ineffective air paths in terms of leaks out of the module, and assisted in reconstruction of a system allowing sufficient air to the animals while insuring encapturing potential escaping particulates. During the development testing, airflow was greatly improved through the cages by placing a coarse mesh screen on the cage top which served as a turning vane for air coming through the inlet plenum of the ECS. Testing with acetic acid smoke revealed that airflow was virtually linear over the entire length of the cage. The improved average 10 CFM airflow through the cages, was in part due to the changed waste tray packing material. Use of Bondina™, charcoal impregnated polyester foam, and Filtrete™, facilitated airflow, eliminated loose charcoal, and maintained 150 micron particle containment, respectively. During SL-3, the use of layers of fiber glass batting, and loose charcoal resulted in inconsistent  $\Delta P$ 's across each cage and loss of charcoal particles

into the cage module. The treatment of all filter materials with phosphoric acid was retained as a standard to prevent odor and eliminate microbial growth.

In addition to LMSC hardware changes, a low crumbing, ten-day duration, wheat based food bar was developed within the SLSPO along with a commercial means of production and microbial resistant.

As further assurance of a "flight-worthy" piece of hardware for SLS-1, the RAHF was extensively tested at ARC. A 14-day biocompatibility test was conducted upon receipt of the unit, followed by System Sensitivity Testing (SST), and an experiment verification test 6 months later (March, 1989). The crew participated in these tests which included demonstration of the SPAF particulate capabilities, odor evaluation, and microbial containment verification. All results were positive. Carbon dioxide levels within the RAHF were also evaluated to insure conformance to less than 0.5%. The tests did reveal that animals would succumb to asphyxiation if there was loss of power and resultant loss of circulating air for periods greater than 45 minutes. This also verified a much tighter sealed unit than SL-3 in which animals could be maintained >4 hours in the absence of power and recirculating air. The second flight RAHF, which was delivered in 1990 and utilized during the Delayed Flight Profile Test, a science control test at KSC, underwent extensive SST. It's profile mimicked that of the first unit, which was integrated into the Spacelab. The SST's characterized the performance of the RAHF including response to high and low fluid loop temperatures, high and low ambient temperatures, half Thermal Electric Unit (TEU) performance. All of this data proved valuable as a diagnostic tool during on the pad and in-flight operations. This data was, in fact utilized as reference, in requesting the lower coolant loop temperature, prior to insertion of animals, on the third launch attempt. Figure 2 illustrates the features of the refurbished RAHF as flown on SLS-1 (contrast to Figure 1).

#### General Purpose Work Station

Following the anomalies of SL-3, the project re-evaluated the General Purpose Work Station's (GPWS) capability for particulate containment. The following activities were implemented during the period 1985-1988 to assure containment:

- Cabinet sealed to NSF-49 Class II standards (contains particles ≤ 150 microns)
- Side access window added to allow entry of small items such as rodent cage without opening the large front window.
- Gauntlet ports added to front and side doors to prevent particulate escape during operation and to keep crew garments clean. Gauntlets are made of Tyvek, a standard medical clean room material. Gauntlets stop at wrist allowing crew to retain surgical gloves required during delicate dissections. Spare gauntlets are installed in stowage, in the event of any tearing.
- Grille covers added inside cabinet to prevent particulates from entering HEPA filter system.

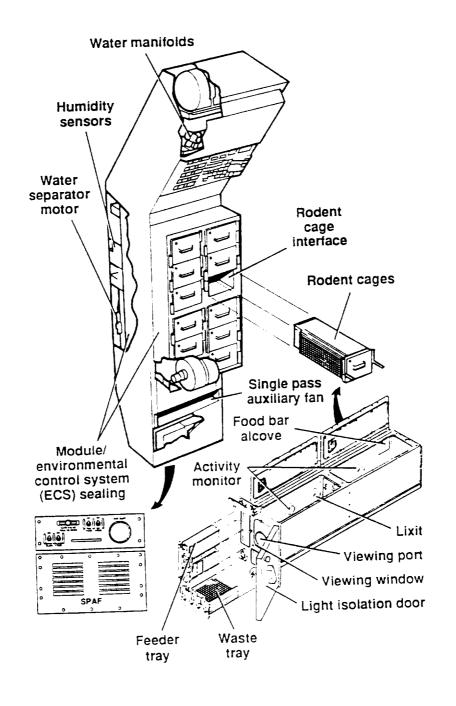


FIGURE 2. SLS-1 Rodent RAHF Configuration

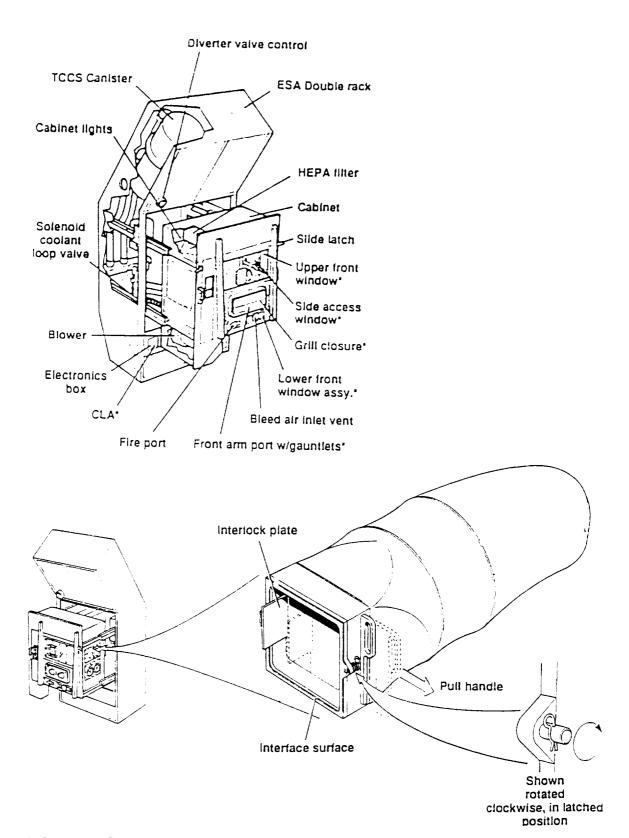


FIGURE 3. General Purpose Work Station & General Purpose Transfer Unit

The GPWS was forwarded to the KSC in 1988, to allow sufficient time for modal testing in the flight rack configuration. As a result of later coupled loads analyses, structural redesign was required which resulted in the following:

- Two overhead stowage lockers eliminated and replaced by single close-out panel.
- Experiment Power Distribution Panel reconfigured to single panel spanning both sides of double rack.
- Bracing at interior corner posts.

Figure 3 illustrates the elements of the GPWS as configured for SLS-1, along with the General Purpose Transfer Unit interfaces.

#### General Purpose Transfer Unit

An auxiliary piece of equipment, the General Purpose Transfer Unit (GPTU), was developed as a result of particulate problems on SL-3. The GPTU was designed to accommodate transfer of rodent cages between the RAHF and GPWS and thus eliminate any potential for release of particulates from the cage to the spacelab environment. The GPTU resembles a wind-sock attached to a lexan box frame. The frame attaches to the RAHF; a cage is pulled into the wind sock and closed off by a door in the lexan frame. The frame is then interfaced to the GPWS. Opening the GPWS side window, opens the lexan frame window and the cage is pulled into the GPWS. RAHF, GPWS, and GPTU interfaces were thoroughly evaluated during the Experiment Verification Test at ARC, prior to flight.

#### Animal Enclosure Modules

The two Animal Enclosure Modules (AEMs) housed five rats each in the mid-deck location. AEMs had been flown on STS 8, 11, 29, and 41 prior to SLS-1. All units are dependent on cabin air and circulation via internal fans for temperature control. The units remain closed during flight and because of their configuration there is no in-flight manipulation of specimens. Observations are through a lexan cover. Food bars are glued to side walls; approximately ~125 square inches of floor space is available. Waste containment and absorption is through use of a phosphoric acid impregnated, charcoal bed/filter pad. Temperature monitoring is via a front faced "meat probe" thermometer or the more recent addition of the Ambient Temperature Recorder (ATR), which is read post flight.

The Ames constructed units were modified from the original General Dynamics unit constructed for the STS student program. Ames units included a 1500 cc watering unit and an automatic light timer.

Several changes were implemented in the SLS-1 AEM:

- Waste filter material changed to resemble that in the RAHF. Resultant weight of AEM decreased ~6 pounds.
- Water box along with in-flight refill unit utilized allowed longer duration flight.
- Ambient temperature recorder (ATR) installed. Results of Scrub #2 ATR playback resulted in pre-flight low temperature conditioning of KSC Biotransport Van (58°) and Level IV carrier unit and request for continuing mid-deck 65° air purge to launch -2 hours.

#### Small Mass Measuring Instrument

The Small Mass Measuring Instrument (SMMI) is a piece of JSC LSLE equipment loaned to ARC. Three units were forwarded to ARC, one of which flew. ARC was required to implement a contract with Southwest Research Institute, the builders of the units, for refurbishment in 1989 since continuous problems were experienced in the stability of the units. Though received as "flight certified" hardware from JSC, extensive additional testing was required by ARC to fulfill all elements of verification as defined in 1986. The SMMI was flown in SLS-1 as a verification of its calibration maintenance capabilities prior to its experiment support use in SLS-2.

#### Refrigerator/Incubator Module

The Refrigerator/Incubator Module (RIM) was procured as an addition to an existing Marshall Space Flight Center (MSFC) contract. MSFC units had been flown earlier in numerous missions since STS 26 supporting microgravity materials experiments. Like MSFC, ARC was required to change out various electrical components and a digital temperature readout was incorporated. For SLS-1, the middeck configured unit was flown in spacelab in the SMIDEX rack configuration. The unit was maintained at 28° and supported the Jellyfish flasks and bags.

#### Miscellaneous Stowage

Various stowage hardware utilized was modified  $commercially \, supplied \, items, e.g., air \, sampler, video \, camera.$ The air sampler is a copy of units utilized previously for microbiological sampling aboard the STS. The agar strips, normally utilized for microbiological sampling were removed. A fine mesh screen, entrapping particles >150 microns, was attached over the mini-centrifugal head. The screens were covered with a solid lid at the conclusion of each sampling and the unit was screwed off the sampler and retained in stowage for observation at mission's end. The video camera was outfitted with a special adapter plate which allowed handling of the jellyfish flasks in a steady mounted position. The jellyfish bagging system was a combination of syringes mounted within sealed bags. Development of equipment supporting the jellyfish experiment (R/IM, video brackets, bagging system) was not started until 1986, when the experiment was manifest aboard SLS-1.

A last piece of stowage, which served as an accessory to the AEMs and the R/IM, was the Ambient Temperature Recorders (ATRs). These units are the size of the ESA type 1 containers, have a wide temperature range, and are battery maintained for several months. The units can also be configured with external probes, if required.

#### RESULTS

#### Research Animal Holding Facility

The RAHF was flown with 19 animals of approximately 250 grams each. One cage compartment (6B) was flown empty because of on pad lixit failure. The other two cage slots, 2A/B and 9A/B contained equipment for the Particulate

Containment Demonstration Test (PCDT). With the exception of the pressure transducer anomaly (detailed under ANOMALIES), the RAHF performed as planned. Figure 4 illustrates the "on pad" T-0 data, which included monitoring of quadrant 1 temperature, humidity 1, TEU coolant inlet temperature, and coolant flow status. The following was observed:

- High quadrant temperature (27°) noted on launch attempt #2. This was attributed to sustained MPE fluid loop temperatures of 21°C. The MPE loop was reduced to 12-14°C and nominal temperature data was received and maintained to L-6 hours.
- Leak alarms noted after launch attempt #2. MVAK technicians were able to reset 4A, 4B. Cage 6B could not be cleared. No animal was placed in that cage slot during launch attempt #3 (19 only animals flown in RAHF). The RAHF was maintained on "ON" condition between launch attempt #2 and #3.

NOTE: Rodents were lowered into the RAHF at approximately launch -29 hours on both launch attempts 2 and 3.

Figure 5 typifies the RF1 and RF2 responses observed throughout the flight and as processed through the ARC ground data compilation. Temperature and humidity matched ground tests, but quadrant 4 was slightly lower than expected. Raising the set point to 25°C (from 24) brought all temperatures to nominal limits. The MPE fluid loop was approximately 12°C.

The water tank pressure transducer failed on flight day (FD) 3. Three activity monitors failed in flight; the data is redundant with water counts. Two experiment computer crashes of approximately 5 hours each interfered with data retrieval. Because of the uncertainty of water consumption versus water availability, the crew was required to add Gel Paks to the cages on FD 8. The following data was retrieved at end of mission and very closely mimicked the data obtained with the second RAHF used during the Delayed Flight Profile Test conducted at the Hangar L, KSC facility 30 days post landing:

- Total condensate collected during the flight
- $= \sim 3.5$  liters
- Microbial analysis of condensate
- = Pseudomonas paucimobilis
- Microbial analysis of water tank
- = No colony forming units
- Total water retrieved from water tank (includes MVAK operations and post-flight micro sample volumes)
- = 3.8 liters

#### General Purpose Work Station

The GPWS was used in flight for performance of the PCDT when both particulates and fluids were released on two different days by two different crew members. In addition, the GPWS was also utilized for:

- -Observation of in-flight release by crewmember of a rat from cage within the GPWS cabinet (FD 7)
- -Addition of gel paks to each rodent cage compartment (FD 8)
- -Fixation of jellyfish specimens within their bag system (FD 9).

All activities with the GPWS were nominal with the exception of several crew observations indicated under ANOMALIES.

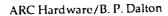
Following the initial particulate dispersion, Dr. Seddon reported particulates settling via the airflow within 20-30 seconds. Initial dispersions resulted in some adherence to interior surfaces which was thought to be due to static attraction. This same condition was not observed during the second dispersion; particulates were readily flipped from surfaces with a plastic bag. A long handled brush will be incorporated in future flight stowage to aid in cleanup.

Post flight microscopic examination of the centrifugal sampler screens collected during both GPWS and RAHF PCDT activities, revealed particulate accumulation under one only condition and on only one screen at a level of <50 microns in size and not exceeding 20 particles/inch. That condition was during the first release and cleanup within the GPWS when the crew failed to adequately clean the interior backside of the GPWS front window and material was entrapped on raising the window. With appropriate cleaning operation, the condition was not repeated during the second particulate release.

The Crew Kit (ping pong ball), implemented at Dr. Seddon's request, proved extremely beneficial in demonstrating airflow patterns and the appropriate window height for retrieval of items without contamination to the spacelab atmosphere.

The PCDT, involving both the RAHF and GPWS was <u>so</u> successful, that the Administrator approved transfer of live rats in their cages from the RAHF to the GPWS for handling within the GPWS. This provided useful insights on animal behavior outside their smaller closed environment (RAHF cage). It also demonstrated debris when the cage was opened in the GPWS since there is no airflow through the cage outside the RAHF. Procedures can be implemented to minimize this release within the GPWS and thus not contaminate any processed samples within the GPWS during SLS-1 experiment activities.

Though the Jellyfish Experiment bagging system was triple contained, the STS Safety Committee requested the GPWS be used for the fixation activities, "... because it was available." The requirement to start up the GPWS and transfer all activities to the cabinet unnecessarily impacted available crew time.



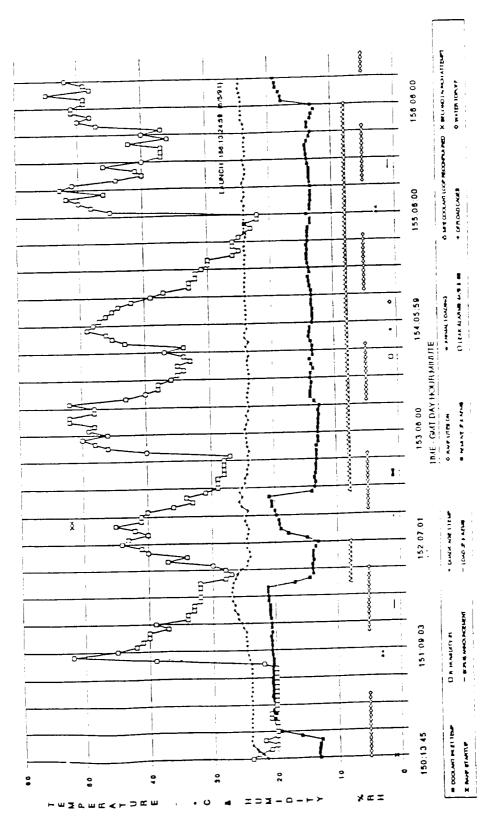
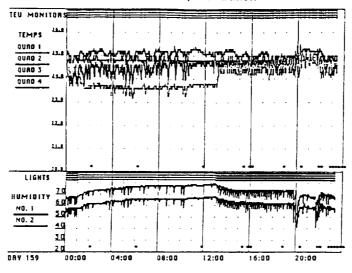
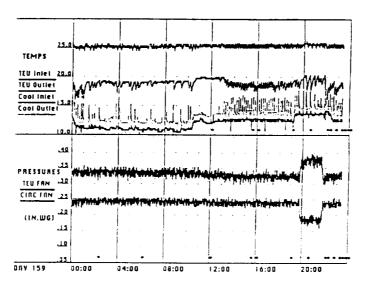


FIGURE 4. On Pad T-0 RAHF Data





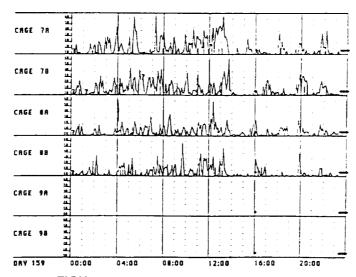


FIGURE 5. RF1 and RF2 Reduced Data

#### Refrigerator/Incubator Module

The R/IM maintained its preset 28° temperature throughout the flight. Figures 6a, 6b, and 6c profile the temperature maintained within the Jellyfish Kits, placed within the R/IM.

#### **Animal Enclosure Modules**

The two units performed nominally. Though lexan windows were extremely soiled by FD 3 (also observed in previous flights) and alarming amounts of debris were viewed floating with animals, the AEM animals appeared well groomed on return and exhibited food consumption, water consumption, and weight gain comparable to that of RAHF animals (Figures 7, and Table 1). Data in Figure 7 and Table 1 is also presented for the Delayed Flight Profile Test (DFPT). Figures & and & profile the ATR data recovered from the flight AEMs.

The In-flight Refill capability allowed use of the AEMs for the extended flight. Normal capacity is limited to a maximum of 6-7 days with the 1500 cc bladder. The addition of the ATRs on the past three flights using AEMs, has provided added insight into flight conditions.

#### Small Mass Measuring Instrument

The performance of the SMMI exceeded expectations. The following data was recovered from operations performed on FD 4 and FD 6:

ITEM	175.21 175.0 175.2 175.1 175.2 175.2 175.3 175.3 175.3	250.21 250.2 250.4 250.1 250.2 250.2 250.2 250.2 250.2 250.1	100.21 + 175.21 275.3 275.1 275.4 275.4 275.4 275.4 275.4 275.3 275.3
AVERAGE	175.2	250.2	275.3

#### **ANOMALIES**

Four anomalies were noted against the ARC hardware during the SLS-1 mission and reviewed by the Robbins Committee. The first three anomalies noted have been closed out by the committee; the fourth remains open for further resolution by ARC:

- Failed lixit, cage 6B
- RAHF leak alarms 4A, 4B, 10B in-flight

- AEM swagelock fitting loose
- RAHF water pressure transducer failure.

The history of these anomalies is as follows:

#### Failed Lixit, Cage 6B

During preflight launch attempt 3 MVAK operations, leak alarms were noted on cage slots 4A, 4B, and 6B. The MVAK technician was able to successfully reset 4A and 4B; 6B did not respond though 180 cc of water was manually drained through the lixit. No animal was placed in the 6B cage slot because of the inoperative lixit.

In conclusion, the problem was due to air in the lines as revealed by post flight lixit testing. Removal of the air resulted in nominal functioning of the lixit along with calibration of that lixit. Corrective action requires burping of the water manifold during the integration process to eliminate air. The procedure was not performed due to schedule conflicts.

There is no effect on subsequent missions provided that appropriate planning is in place, e.g., procedure to be included in Ground Integration Requirements Document, and correct integration burping procedures are scheduled and implemented. For those leak alarms occurring as a result of rapid water consumption by the rat or bumping against the lixit, ARC is attempting to design a monitoring system for use pre-flight which will allow tracking of water counts and master reset of leak alarms remotely from the Launch Control Center console.

#### RAHF Leak Alarms, 4A, 4B, 10B In-flight

Leak alarms occurred in cage slots 4A and 4B and were discovered on FD 1 during spacelab activation. A leak alarm also occurred in cage slot 10B on FD 2. The RAHF water system is designed to shut the lixit off if greater than 8 counts are received in an 8 second period. During the ARC biocompatibility and verification tests, 3 leak alarms were experienced during each test. In conclusion, the system performed nominally; to change the system would be counteractive to required safety constraints. No corrective action should be implemented.

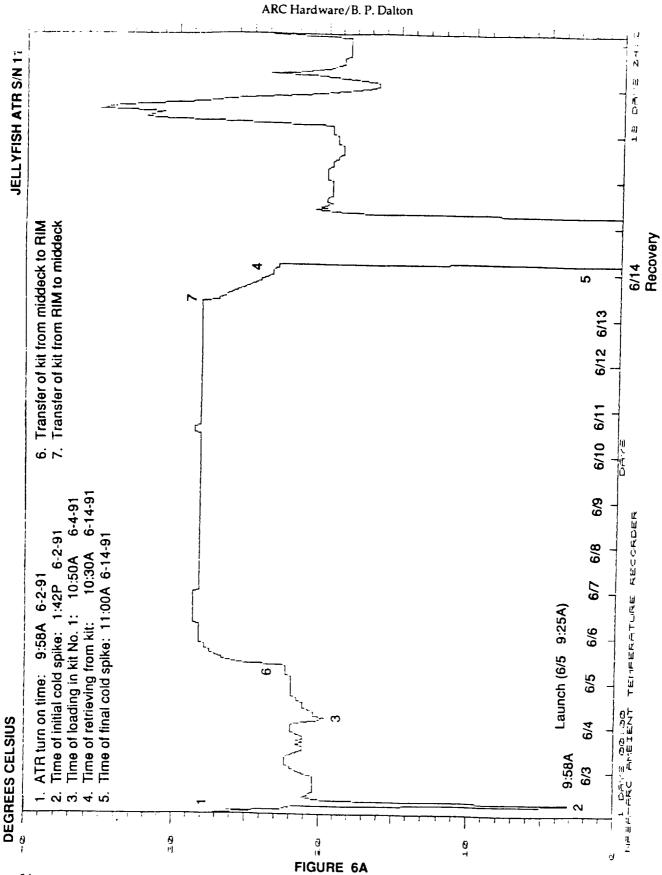
#### **AEM Swagelock Fitting Loose**

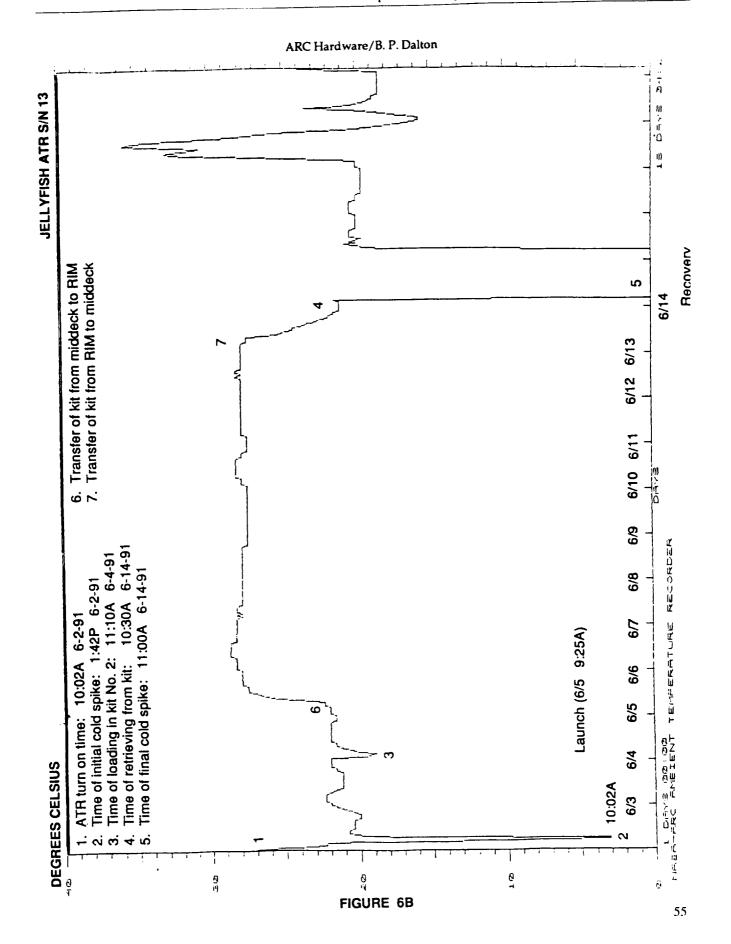
On FD 5 the crew was required to refill the AEMs. AEM #1 was filled nominally. AEM #2 filling was started and a water leak appeared around the swage fitting on the refill lines. The maximum volume of water released, as reported by the crew, was 0.25-0.50 cc. The crew was able to hand-tighten the

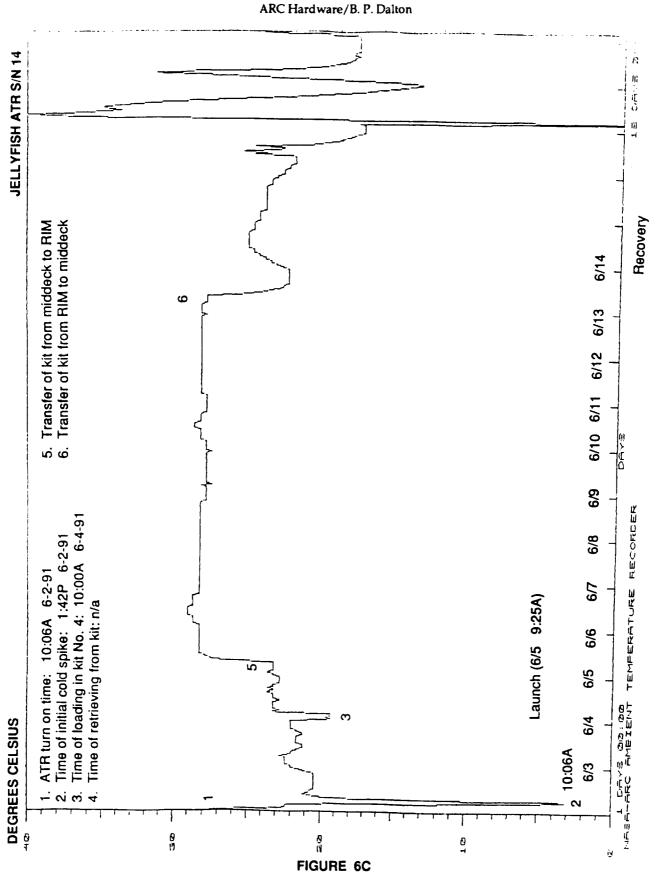
TABLE 1. Food and Water Consumption for the RAHF and AEM Animals

GROUP	FOOD CONS	UMPTION/RAT/DAY	WATER CONSUMPTION/RAT/DAY
RAHF Flight	28.4 ± 2.4	grams	33.5* ml
AEM Flight	27.2	grams	40.5 ml
RAHF DFPT	28.3 ± 3.4	grams	27.1° ml
AEM DFPT	29.3	grams	47.6 ml
		and the state of the Co	EL Dak additions

<sup>\*</sup>Preliminary estimate. Does not include adjustments for GEL Pak additions







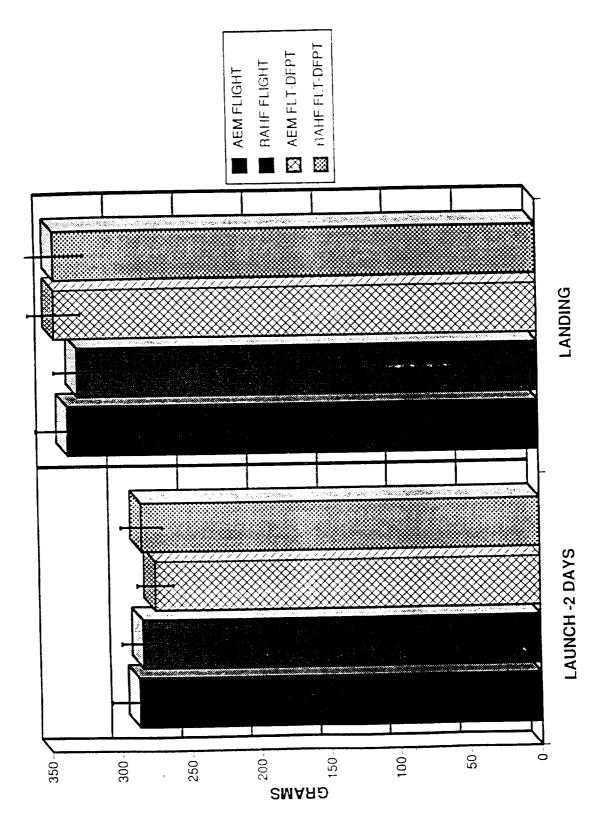
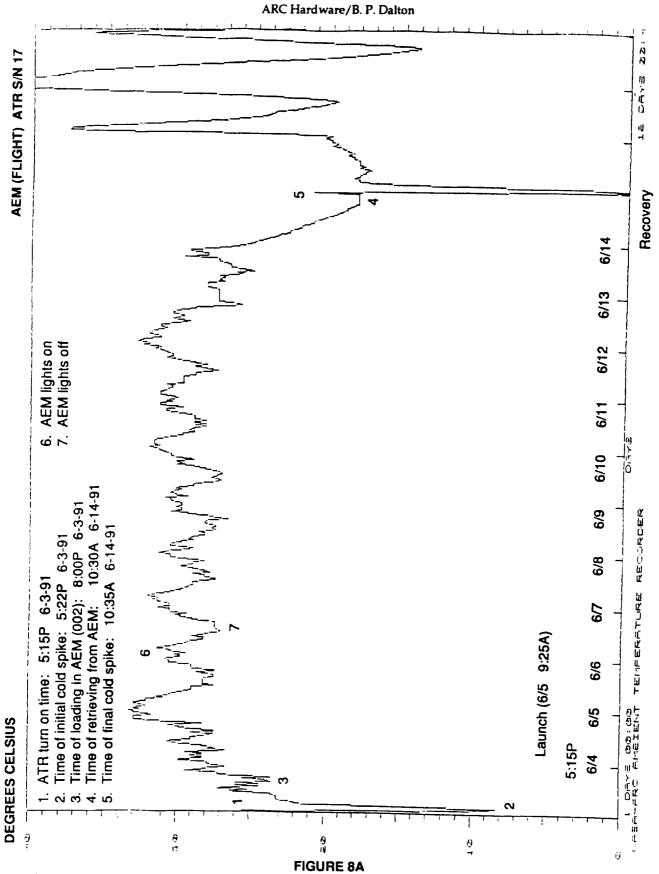
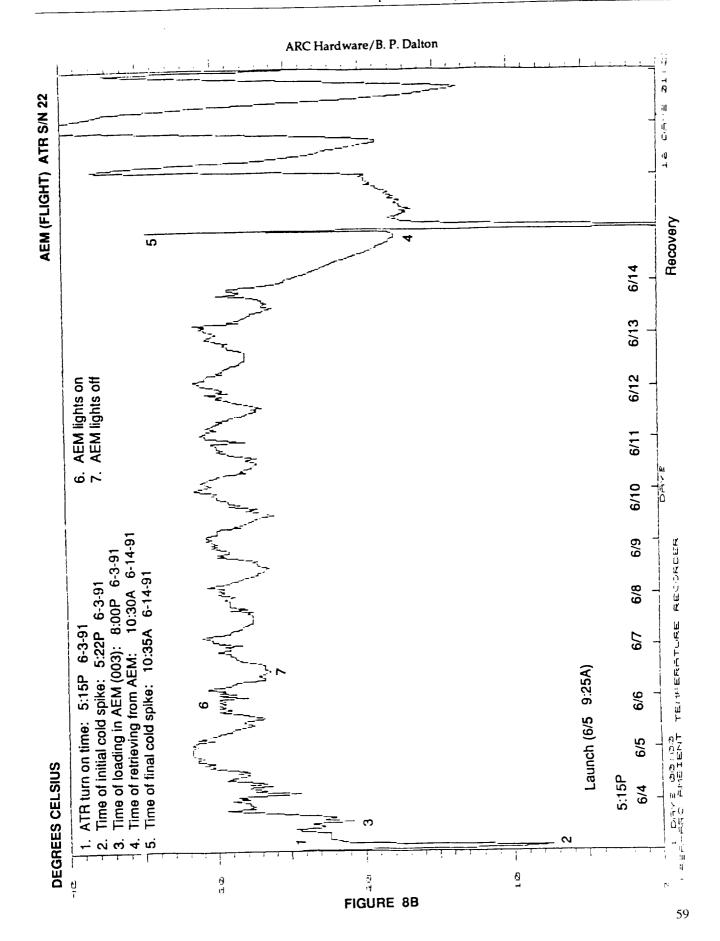


FIGURE 7. Rodent Body Weights





fitting completely and eliminated any leaks. The second filling on FD 8 was without incident.

In conclusion, there was inappropriate inspection of lines and fittings during the preflight preparations. Appropriate inspection points in assembly procedures will eliminate the problem The corrective action involves evaluating preflight assembly and processing procedures and inspection lines to insure proper hardware configuration.

#### **RAHF Water Pressure Transducer Failure**

The RAHF water pressure transducer is a high reliability part. This pressure transducer operated nominally during all functional testing both at ARC and KSC, and through all testing and refill operations performed during Levels IV through I (on the pad). On FD 3, the RAHF's transmitted readings of water tank pressure went from 36.8 psi to \*\*\*\*\*. Evaluation of the "raw voltage" showed a constant reading of 102 psi, which is full scale.

As part of the failure analyses, the RAHF and other systems were tested outside the spacelab, but in the flight rack configuration post flight:

- RAHF powered with ground support equipment. The transducer read 22-18 psi, which matched the 3.4 liter volume left in the tank.
- Flight RAU tested with ground unit tester, which applied voltage through the unit and verified channel response. All elements performed nominally.
- RAHF/RAU interface was tested by applying GSE power to determine if translational voltage from transducer to RAU (or reverse) could have resulted in failed readings and the resultant 102 psi voltage indication. Both the RAHF transducer and the RAU performed nominally.

In conclusion, we currently have an unexplained anomaly. The RAHF was returned to ARC from KSC the week of November 8, 1991. Testing is continuing to resolve the issue prior to SLS-2 use. ARC will continue to use high rel parts and will install a manual gauge for direct readout, in the event a similar anomaly occurs during SLS-2.

#### Other Issues

The following lists other issues referenced during crew debriefings and various reports. A brief response follows:

- PCDT particles stuck in GPWS grilles
   Care should be observed not to push large items through grilles. Items, larger than the grille width, were not intended to be pushed through the grilles.
- PCDT particles stick to GPWS door
   A long handled cleaning brush will be installed in SLS-2 stowage to facilitate cleaning in the corners, crevices, and on inside of door face.
- GPWS rails bind and GPTU/GPWS mating
   The rails on the CPWS side window used in SLS-2 will be reworked. SL-J uses a plain window.

Dirty velcro in GPWS

The project will investigate use of a double backed velcro which may be easily replaced inflight. The brush referenced above may also facilitate cleaning velcro.

Gauntlets limit visibility

The crew did not use the garters provided; SL-J has chosen to use a rubber band to curtail ballooning effect of gauntlets. ARC is investigating elastic shirring down gauntlet side to minimize ballooning.

- RAHF adapter rails were loose
   Detents will be tightened prior to SLS-2 with positive latch.
- Slide valve on RAHF SPAF
   The RAHF office will investigate a variable flow capability on the SPAF to reduce the potential for feces from cage front waste compartment from drifting to back compartment during SPAF activation.
- Tight foam around AEM Refill Unit
   This problem has been reported in previous flights. The recommendation is more project interaction with Boeing FEPAC along with "fit checks" prior to shipment of foam inserts to KSC.
- Heightened AEM preflight temperatures
   ARC has implemented procedures to circumvent
   elevated temperatures in the AEM including
   cooling the BTV, purging the mid-deck with 65°
   air to as late as possible prior to launch, utilizing
   only 1/2 bank of lights. Prior to the use of the
   ATRs in the AEMs, these pre-flight elevated
   temperatures were not "apparent".
- GPWS phase imbalance

The GPWS was retested with QA witness during SL-J integration. There is no phase imbalance.

GPWS low flow light

The "LO FLO" light was activated on the last flight day during the jellyfish fixation activities in the GPWS. Two possibilities exist to explain this anomaly:

- -It is not clear if the grille closures were completely opened.
- -Sufficient particulates may have been suspended in the system to block the system.

The GPWS was activated on return to 1G, the grille closures, though difficult to open, were operated in the "OPEN" position and the unit performed nominally.

All Problem Reports (PRs) and Field Engineering Changes (FECs) generated at KSC are being reviewed prior to refurbishment of any hardware utilized in succeeding missions.

# SPACELAB LIFE SCIENCES 1 AMES RESEARCH CENTER TRAINING

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Received December 4, 1991

HE FOLLOWING INFORMATION addresses training from September 1987 until the launch of STS-40 on June 5th 1991. As of August 1988, MMO was distributing schedules showing a June 1990 launch date. Consequently, training schedules reflected that July 1988 was launch minus 23 months (L-23 months) and ARC was preparing to coordinate training for the SLS-1 Payload crew. The generic training template used by ARC to schedule training was difficult to follow due to several launch slips and hardware and crew unavailability.

It should be noted that the Payload Crew had already begun training on SL-4 experiments in the Fall of 1983. When training resumed in the Fall of 1987, the original SL-4 payload had been reduced to hardware verification of the RAHF, RAHF Adapter, GPWS, GPTU, and SMMI. (RAHF, GPWS, and GPTU verification was to be accomplished through the Particulate Containment Demonstration Test). In addition, crew inflight activities concerned with RAHF/AEM Rodent Health Observations, AEM Water Refill, Jellyfish Inducement and Fixation, and Jellyfish Filming were scheduled.

#### **ARC TRAINING**

The Ames Research Center (ARC) mission dependent training is divided into timed phases: Orientation, Task, Phase, Project Integrated, Mission Integrated, and Proficiency Training. Every component of each experiment and associated hardware is subject to the same basic training template. This approach provides an ideal working model as each successive training session builds knowledge gained from the previous training session until proficiency on Integrated Payload Procedures is achieved.

The obstacles that greatly affected the training program were hardware availability, changing inflight requirements, and launch slips. With every launch adjustment, Mission Specialist support fluctuated and required additional resources to bring all individuals to a similar level of proficiency. On the other side of the coin, hardware development and verification were often not in sync with hardware availability requirements to support in the training of the payload crew and to assist in procedural development.

#### Orientation Training

The first exposure to orientation training, in the then present reincarnation of the SL-4 experiments, occurred in September of 1987 and was finally completed in February of 1989.

Training was accomplished at either ARC facilities or at the Principal Investigator's (PI's) lab (for the Jellyfish Experiment). The crew received orientation to the ARC complement of rack mounted hardware, i.e., RAHF, GPWS, and SMMI, Jellyfish experiment and associated hardware, and the middeck stowed AEM's. The crew also received an orientation on the Cardiovascular animals, which at this time were to be housed in an AEM. Interspersed within this window was a training session, May of 1988, to review PCDT activities and associated tasks to be performed on a KC-135 flight in June of 1988.

Approximately 47 Orientation training hours were accomplished for each crew member during this interval of training. This does not include the additional hours each crew member spent prior to May 1988 nor the additional hours required to review training materials prior to the start of the scheduled training session.

#### Task Training

During Task Training, the payload crew became proficient in all aspects of the experiment objectives through intensive and in-depth lectures on Experiment Unique Hardware (EUH), stowed items, discussion of procedures, and through "hands-on" training with specimens and available experiment hardware. Based on the overall launch schedule and the availability of the hardware and the crew, task training was often accomplished together with orientation training.

Task training on PCDT activities was provided on three training dates (September 1987, November 1987, and January 1989). The payload crew also received training on the Jellyfish experiment, SMMI, GPWS, and RAHF. A total of approximately 49 hours were accumulated in support of Task Training.

#### Phase Training

Phase training was designed to allow the crew the opportunity to complete enough repetitions of the experiment so the crew member would be able to complete the experiment procedures at a defined level of time proficiency. Training was to have utilized the experiment operating procedures, payload specific hardware, and stowage items. This training opportunity was also to provide the crew with a level of proficiency which would guarantee a meaningful participation in the Experiment Verification Test. The crew logged approximately 37 hours during this portion of the training and it was accomplished over a period of two years and 3 training opportunities.

#### Project Integrated Training

The objective of Crew Training during the SLS-1 EVT (February 28-March 8, 1989) was to conduct Project Integrated training of the payload crew members. They were to perform all ARC in-flight activities to assist in validation of the SLS-1 timeline. Although the crew was familiar with the ARC payload, this EVT was the first time they were to combine the tasks into operational procedures with most of the flight hardware and stowage items available for their use.

Unfortunately, the Payload Crew Mission Specialists were not available to support the EVT while the primes and backup Payload Specialists attended and participated in a large number of the in-flight sessions. Their participation covered approximately 40 hours of the total 72 hour execute shift.

#### MISSION MANAGEMENT OFFICE TRAINING

#### Mission Integrated Training Simulations (MITS)

The objectives of MITS are two-fold; they allow the crew to develop their proficiency to a level of performance where they can successfully perform all the payload activities within the mission timeline and they allow the Payload Operations Control Center (POCC) cadre and PED-support the opportunity to rehearse inflight ground protocols. MITS are similar to Project Integrated Training, but include timeline performance of all mission experiments and other activities necessary to carry out the mission.

MITS occurred within a fully integrated spacelab mockup and was supported by ARC Training at every session. Integration of the Building (Bldg) 36 mockup began June 1989. Confusion existed initially due to the fact that ARC hardware was mockup fidelity and not flight; the level of JSC Bldg.36 Quality Assurance was sometimes inappropriate. Training included not only nominal operations but also malfunction training.

The SLS-1 payload had the unique opportunity of participating in ten simulations with the POCC cadre (including Mission Management Organization (MMO) and Payload Experiment Developer (PED) support personnel). In addition five Joint Integrated Training/Simulations (JIS) were scheduled with POCC Cadre at MSFC, Mission Control Personnel at JSC, and the crew traveling between the Bldg. 36 spacelab mockup, the Bldg. 9 middeck mockup and the Bldg. 5 simulators. Each of these training opportunities simulated different start and stop times on the overall mission timeline. This required that the mockup, including stowage, be configured to simulate the mockup as it would appear at the start time of the simulation for that particular flight day (FD).

Payload Crew members (i.e., Mission Specialists Rhea Seddon, MS3; Jim Bagian, MS1; and Payload Specialists Drew Gaffney, PS1; and Millie Hughes-Fulford, PS2; participated in Mission Integrated Training. Bob Phillips, who was identified as the Alternate Payload Specialist, supported all training simulations by serving as the voice interface between the crew and the POCC cadre. The Orbiter Crew, i.e., Bryan O'Conner, Commander; Sid Gutierrez, Pilot; and Tammy Jernigan, MS2, was selected later than the

Payload Crew and as such their participation came later in the flow of these events. (Note, these additional assignments required that ARC provided orientation to the ARC payload as well as exposure to the hard ware and in-depth training on any ARC experiments they were to perform in flight).

MITS training dates and Flight Days (FD) simulated were as follows:

MITS #1	July 26-27, 1989	FD1 (Spacelab Activation)
MITS #2	August 23-25, 1989	FD2-4
MITS #3	October 17-19, 1989	FD4-7
MITS #4	December 5-8, 1989	FD2-5
MITS #5	January 17-18, 1990	FD3-4
MITS #6	March 12-16, 1990	FD4-6, FD7-8
MITS #7	April 17-19, 1990	FD1-3
MITS #8	September 24-25, 1990	FD1
MITS #9	November 26-28, 1990	FD4-5
MITS #10	February 12, 1991	FD1

JITS training dates and Flight Days simulated were as follows:

Pre-JIS	February 20-22, 1991	Simulation for POCC Cadre Only Alternate Payload Specialist
JIS #1	March 20, 1991	FD1 (Ascent/ Activation)
JIS #2	April 2-3, 1991	FD4
JIS #3	April 16-17, 1991	FD1-2
JIS #4	May 3, 1991	FD9 (Deorbit)

#### LESSONS LEARNED

The following lessons learned are an attempt to address some of the difficulties associated with training a crew and to demonstrate that inflight operations should be relegated to a higher level of priority during payload development and maturation. While there may be many more "lessons learned" that may contribute to a successful payload, these are presented from an operations standpoint. Delivery of the hardware to meet integration is highly critical, but it is the success or failure of the inflight operations that will be remembered and used to determine the outcome of a mission.

#### Training and Procedure Development

- Hardware must be available to support procedure development and training, but is in conflict with hardware verification and delivery dates to STS.
- Higher fidelity mockups of training hardware are required to support Mission Integrated Training Simulations.
- Spacelab mockup used to support Mission Integrated Training Simulations must be configured correctly and validated prior to the onset of this phase of training.
- Procedure development requires the use of high fidelity flight-like hardware many months prior than the present payload development schedule allows (Payload considered mature and frozen at CDR, ~L-18 months

- but crew begins training between L-24 and L-18 months, consequently, procedure validation using flight-like hardware cannot occur early.
- Month by month launch delays prolong the training program such that skills are dampened and performance quality decreases.
- Crew must be exposed to procedures that have been correctly formatted into a preliminary inflight version at the onset of integrated training.
- Preliminary inflight documentation must be available to support Mission integrated Training.
- Clear and detailed science and engineering requirements must be provided that address crew operations covering the range of activities from photo/filming to inflight data collection.
- Every activity timelined concurrently or on either side of an ARC experiment must be performed during a simulation.
- Possible stowage interference with other payload experiments must be determined when ARC experiments are performed.

- Changes to any procedures must be completed well in advance of L-1 month. MMO needs to work their procedure delivery schedule much differently and the Project must make sure that all procedure verification is done early in the documentation cycle.
- ARC must verify stowage and foam fit checks while foam is in its locker, regardless if MMO is responsible for fabricating the foam.
- Stowage closeout pictures and hardware switch panels should be taken for crew update/familiarization and also to POCC inflight activities.
- Individually wrapped items should be repackaged into groupings to avoid excessive garbage generation.
- SMMI weight Kit needs to reworked, i.e., foam needs a snugger fit while in the kit.
- Labelling of items should be considered as high a priority as the actual hardware concerns.
- Procedures sent to inflight crew should always be in the same format they are familiar with seeing. The ground should not be providing ground or MVAK procedures since the crew has probably never seen or worked with this version of the procedures. There should only be one source for the procedures.

Greater details of Lessons Learned affecting PED elements are detailed in the ARC SLS-1 90 Day Report.

# ARC SPACE LIFE SCIENCES ONE (SLS-1) BASELINE DATA COLLECTION

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Received December 19, 1991

#### **OVERVIEW**

HE INFORMATION PRESENT in this section is intended to provide a brief description of the science activities immediately pre- and postflight. Data presented has been restricted to rodent maintenance data. General information is also provided on dissection activities including samples taken, and sample shipment summaries. Detailed analysis of individual groups as well as comparisons of rodents in the RAHF vs. the AEMs will be presented in the one year report.

Off-site activities began in earnest on April 1, 1991 with preflight preparation of the Payload Receiving Facility (PRF) at Dryden and continued at a hectic pace through lab deactivation at KSC following the completion of the Delayed Flight Profile Test (DFPT) July 31, 1991.

An exceptional level of effort was required by the entire SLS-1 team through the duration of the off-site activities in order to successfully complete all of the required tasks. This sustained effort, coupled with extended travel, had a significant impact on the team as demonstrated by fatigue, loss of productivity, and low moral for periods of time. Some of this overburden was due to underestimation of required manpower for planned tasks, but the bulk of the additional effort was required for unanticipated tasks. Hopefully, we can pass our off-site experience to future payloads so they can better plan their manpower requirements.

The success of SLS-1 was not the result of any single group, but rather the combined effort of the entire ARC SLS-1 team, PI teams, and off-site support personnel at KSC and Dryden. While the combined effort was successful, it was not without difficulties.

#### Off-Site Lessons Learned

High fidelity dry runs at both KSC and Dryden (not discused in detail in this section) were extremely useful for identifying and resolving issues prior to flight and should be mandatory for all payloads.

ARC, KSC, and Dryden regulations and requirements regarding procedural aspects of safety, shipping, and waste disposal were in many cases very different and added a layer of confusion that translated into a very significant manpower effort to resolve. Solutions that were developed for SLS-1 should reduce, but not eliminate, these problems for future payloads.

PI requirements to HIRD and GSRD translation were an extremely tedious task and tracking the status of the individual items through KSC and/or Dryden was difficult, if not impossible at times. Common software should be adopted by KSC and ARC to make transfer of information from the HIRD to the GSRD transparent. ARC should procure and track all chemicals and critical lab supplies.

The administrative load of coordinating travel and lodging for close to 90 members of the ARC SLS-1 team took valuable timeaway from many primary tasks. Future payloads should identify additional administrative manpower to reduce this burden, which detracts from required pre/postflight experiment activities.

#### EXPERIMENTAL DESIGN

#### RODENT MAINTENANCE

#### Receipt

Seven of the eight primary investigations from the Ames Research Center Payload utilized the rat (*Rattus norvegicus*) as the model for study. For each week of launch attempts, 181 male rats of Sprague-Dawley strain were ordered from the vendor (Taconic Farms).

After 10% of the group was removed by the vendor per ARC request for microbiology and necropsy (AnMed Labs), 163 rats were shipped to Kennedy Space Center. Each shipment of rodents was escorted by ARC personnel to insure proper transport conditions and to note any anomalies.

Group Designation	Arrived at Hangar L
Launch Nominal Group Launch Contingency Group 1 Launch Contingency Group 2 Launch Contingency Group 3 Launch Contingency Group 4 Delayed Flight Profile Test Group	April 24 May 1 May 8 May 15 May 29 June 12
	,=: :=

The 163 rats were then received at the portable clean room at Hangar L, where the rats were ear tagged, weighed and provided food and water ad lib. Upon receipt, KSC elected to sample an additional 10% of the animals for microbiology (University of Miami labs). The rodents were all initially kept single housed in standard clear plexiglass vivarium cages

with microisolator lids and corn cob type bedding.

All rodents were 5 weeks old and approximately 90-110 gms at receipt.

## Preflight

The rats were transfered immediately from the portable clean room to the Hangar L Animal Care Section where each group (LNG, LCG1, LCG2, etc.) was confined to a separate room. Body weights, and food and water consumption were recorded every three days. Cages were changed out every six days when the rats were single housed and every three days once the animals were group housed. KSC performed tests for ammonia levels in the cages and all levels were below NIH standards. Rodent health observations were performed on a daily basis by the Hangar L Animal Care Technician in charge (Ramona Bober).

In support of daily rodent maintenance, ARC provided a minimum of one person to oversee data collection which was recorded by hand. Hangar L supplied 4-8 persons per day depending on the number of rodent groups requiring attention.

Each initial group of 163 rats went through several culls where 10% of the group was removed in order to achieve a homogeneous population for final flight selection. The culled animals were utilized for dissection practice, hematology blood donation, or spontaneous use such as the testing of Heparin stock.

All animals that were not utilized were euthanized as soon as the determination was made that they were no longer needed. Animals that were injected with isotopes were disposed of as radioactive waste, in accordance with NASA radioactive waste disposal guidelines. All non-radioactive carcasses were frozen and given to University of Florida Zoological and Wildlife Vet Clinic or The Audubon Bird of Prey Program at Maitland FL.

On L-13 flight candidate rats were selected to be AEM or RAHF candidates (group housed or single housed). All flight candidate rats (123) at L-13 were placed on flight food bar diets.

Actual flight and ground control groups were selected on L-3 days, just prior to cage loading and turnover. Selection was based on the following criteria:

- Animal health as determined from daily observations and ARC veterinarian reports.
- 2) Rodent weights and weight gain history.
- Hematology team comments on usefulness of each rat for injections or blood draw.
- Comments recorded during the injection of bone markers.
- General animal behavior or anomalies.

Rats were then randomly placed in groups, some groups designated for nominal launch attempts and others for scrub attempts. Each overall group received at KSC could support 2 launch attempts (nominal launch attempt and 96 hour launch attempt).

The rodent group selection process is better clarified in Figure 1, SLS-1 Rat Selection Flow Chart.

Rats were loaded into the RAHF cages at L-33 hours (12 midnight) in the portable clean room. Group housed rats were loaded into AEMs immediately following RAHF cage loading, about L-32.5 hours (12:30am). The RAHF cages were then loaded into the module at about L-29 hours and the AEMs loaded into the middeck at approximately L-15 hours.

## Inflight

Once the flight hardware had been turned over to level IV personnel, the ground AEM units (S/N 001 and 004) were transfered to the Animal Care Section. The ground AEMs received observations every day of flight. The remaining vivarium housed rats, including RAHF ground control rats were left in the Animal Care Section where food and water weights were recorded on a daily basis along with an observation.

On L+2 days, all vivarium caged rats were placed into cardboard rodent shippers with food and water. The single housed rodents were loaded 4 to a shipper, the group housed ten to a shipper. These shippers were then loaded onto the charter plane along with the ground control AEMs and transported to Dryden's Payload Receiving Facility. At the PRF the rats were then replaced into vivarium cages with food and water. Daily rodent maintenance continued with food, water, and observations on the RAHF ground controls.

#### Recovery

Upon recovery, the ground control AEMs were brought to the receiving trailer (see layout) opened, the rats were removed one at a time, observed by the ARC Veterinarian and weighed. Each rat was then placed into a clear aquarium cage where it was photographed and videotaped for 20-30 seconds. If the rat was to be dissected on recovery day it was placed into a vivarium cage with bedding and without food or water and immediately sent into the dissection flow. If the rat was not to be dissected until R+9 days, then the rat was placed into a vivarium cage with bedding and with food and water and sent immediately to the hematology operations area.

This recovery process occured for all groups in the following order:

AEM ground controls AEM flight RAHF flight RAHF vivarium ground controls

See Figure 2, SLS-1 RAHF Cage Assignments, for a graphic representation of the RAHF rodent number cage assignments.

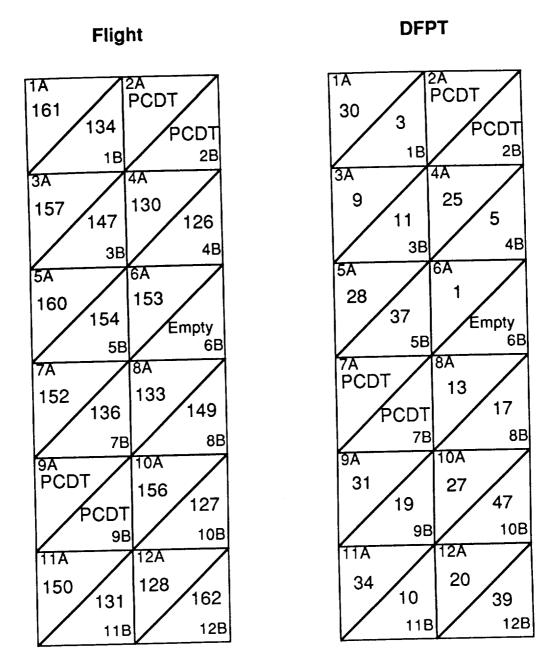
### Postflight

Those rats to be dissected on R+9 days were either group housed (AEM) or single housed (RAHF) in vivarium cages. These rats had body weights and food and water weights recorded every day. In addition, these rats underwent injections and blood draws per the Hematology schedule.

## **Ground Control** 5 backups P P 123 Flight Candidates 14 Ground support (Proficiency pool) AEM Launch Group B Supports 72 and 96 Hour Hold Launch Attempts Group Housed Flight Candidates 10 AEM Launch Group B Second Selection Selection 10% cull L-16 DAYS 5 backups Launch Group A Supports Nominal and 24 Hour Hold Launch Attempt 10 AEM Launch Group A L+0 Bessi control 15 Ground support (Proficiency pool) 138 Mission Candidates First Selection 10% cull L-25 DAYS 10 Rodents Hematology Expt. Donors 20 RAMF Vivarium Control 10 L+0 Basel control KSC Receipt 163 Nominal Mission Pool 4 backups 10% Receipt Micro Launch Group B Supports 72 and 96 Hour Hold Launch Attempts 20 RAHF Launch Group B 78 Singly Housed Pight Candidates Escorted Transport L-28 DAYS 18 Rodents to Anmed for Micro/Necropsy 4 backups RAHE Launch Group A Supports Nominal and 24 Hour Hold Launch Attempt Selection at Vendor 181 Rodents 100gm 20 RAHF Leunch Group A

FIGURE 1. SLS-1 Rat Selection Flow Chart

# **SLS-1 RAHF Cage Assignments**



<sup>&</sup>quot;A" cages are located in the front of the RAHF

<sup>&</sup>quot;B" cages are located behind the "A" cages

On R+9 days the rats were removed from their cages as per the R+0 protocol. Once the rat had been weighed and videotaped it was placed into a vivarium cage <u>without</u> food or water.

#### **DFPT**

The rodent maintenance schedule for DFPT mirrored the flight schedule with the exception that all DFPT operations occurred in the Hangar L Animal Care Section. No L+2 transfer was simulated during the DFPT.

#### Lessons Learned

- A payload of the size of SLS-1 must have a dedicated Rodent Maintenance Team. Management of large-scale rodent maintenance is a critical, full time job, requiring round-the-clock attention.
- A complete rodent census must be available at all times.
   This requires daily attention.
- While the total number of rats utilized should be reduced whenever possible, lack of sufficient rodent groups will jeopardize the entire payload. Always plan for contingencies.
- A rodent disposition plan must be prepared well in advance of the flight and must be agreed to by all responsible parties.
- It must be possible to verify and manipulate rodent maintenancedata on a real-time basis. On large payloads this is a major effort. This should be worked into the payload Data Management Plan and supported with adequate manpower.
- Rodent selection criteria must be predefined and incorporate PI input.
- Standard protocols should be set for all post-flight rodent videotaping. This is a valuable piece of data and should be acquired for every ARC rodent flight possible.

#### RODENT MAINTENANCE DATA

A massive amount of rodent maintenance data was generated during SLS-1 preflight, inflight, and postflight activities. A majority of this data has been reviewed and released to the

principle investigators, however, the process is quite cumbersome and slow. Plans are being developed for improving the efficiency of data acquisition and transfer on future flights.

Following is a list of raw data generated during SLS-1 rodent activities. Again, these data are currently being scrubbed for accuracy. Data is available from both the flight and the Delayed Flight Profile Test.

- Daily rodent body weights
- Daily rodent food consumption
- Daily rodent water consumption
- Daily rodent health check/observations
- Major organ weights from specimens taken at L+0, R+0, and R+9 days
- Video of rodent movement at R+0, R+9 days

Table 1 identifies rodent food and water consumption information for critical times during the SLS-1 flight period.

All daily means for both food and water consumption are within normal ranges. There does appear to be a greater rate of water consumption in the AEM vs. the RAHF, however, it is not clear whether this is due to an increase in consumption by AEM rats or due to an increase in the amount of water loss resulting from inadvertent lixit activation.

Water consumption for the RAHF is based on preliminary preflight and postflight tank volumes. Per rodent consumption is currently being extrapolated from daily single-rat lixit counts and pre/postflight lixit calibrations.

Table 2 lists rodent body weights and average daily weight gain for the flight period.

There was no significant difference between Flight and DFPT SIM-flight groups at launch or landing in terms of mean body weights. There was a statistically significant difference between the Flight and DFPT SIM-flight groups in terms of body weight gain over the flight period. Again, there was no significant difference between Flight and DFPT SIM-Flight rats in terms of food or water consumption, and all general health criteria were good for all groups. It would seem therefore that the difference in weight gain between Flight and DFPT SIM-Flight groups reflects an alteration in the level of metabolism experienced by spaceflown rats. Data from other SLS-1 rodent investigators will help clarify this phenomena.

TABLE 1. Rodent Food and Water Consumption During Flight

Group	Food Consumption/rat/day	Water Consumption/rat/day
RAHF Flight	$28.4 \pm 2.4$ grams	*33.5 ml
AEM Flight	27.2 grams	40.5 ml
RAHF DFPT SIM Flight	$28.3 \pm 3.4$ grams	*27.1 ml
AEM DFPT SIM Flight	29.3 grams	47.6 ml

<sup>\*</sup>Preliminary estimate. Does not include adjustments for Gel PAK additions.

TABLE 2. Rodent Body Weights and Growth for the Flight Period

Group	Loading (L-2 days)	<u>Landing</u>	Weight gain/rat/day
RAHF Flight	$284.1 \pm 15.3  \mathrm{gms}$	$328.5 \pm 16.7  \text{gms}$	$4.23 \pm .88 \text{ gms*}$
AEM Flight	$287.5 \pm 19.8 \text{ gms}$	$335.9 \pm 23.0~\text{gms}$	4.61 ± .99 gms*
RAHF DFPT	$284.5 \pm 15.5  \mathrm{gms}$	$344.5 \pm 27.1 \text{ gms}$	$5.71 \pm 1.6  \text{gms}$
AEM DFPT	275.0 ± 12.9 gms	$344.7 \pm 18.8 \text{ gms}$	6.64 ± 1.1 gms

<sup>\*</sup>Significantly different from ground controls at p≤.05.

# ARC Space Life Sciences One (SLS-1) BIOSPECIMEN SHARING PROGRAM

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## **OVERVIEW**

**Evolution of Tissue Sharing on SLS-1** 

S THE ANIMAL EXPERIMENT manifest for SLS-1 A S THE ANIMAL EXTENSIVE (SL-4) developed, it became apparent that the the most efficient way to maximize the use of the limited number of animals available was to integrate the experiment requirements and develop a tissue sharing plan. This original sharing plan was restricted to the Principal Investigators selected from the Announcement of Opportunity in 1978. This plan was modified on numerous occasions to meet changes in payload hardware configurations (2 rodent RAHFs, 2 AEMs, and 2 AEMs + 1 RAHF). When the sharing plan matured and the hard ware configuration was finalized with 2 AEMs and 1 Rodent RAHF, numerous potentially valuable tissues were identified that would not be utilized by the Principal Investigators.

A Biospecimen Sharing Program (BSP) was developed to insure that these valuable tissue samples could be distributed to appropriate investigators. The primary objective of this program was to maximize scientific return from the specimens flown on SLS-1 with the secondary objective of encouraging broader participation of the research community in the Life Sciences Flight Experiments Program. Acceptance of experiments for the SLS-1 BSP was based on: 1) scientific merit; 2) compatibility of the requested tissues with prime SLS-1 experiments; 3) the dissection team's ability to collect and distribute tissues as per the requirements of the investigator; and 4) where relevant, concurrence of the primary principal investigator.

The SLS-1 BSP was initially limited to an extension of the joint U.S./U.S.S.R. studies conducted on the U.S.S.R. biosatellite Cosmos flights 782, 936,1129,1514,1667 and 1887. Seventeen Soviet experiments were accepted as part of U.S./ U.S.S.R. joint working meeting in 1989. As part of the agreement Soviet experiments were limited to tissues from the ten rats flown in the AEMs. In addition all tissues were to be collected and processed by a U.S. dissection team trained in the U.S.S.R. protocols. The objectives of these U.S.S.R.proposed experiments were to investigate metabolic, structural and functional changes in the rat body under the influence of a short-term exposure to microgravity. The biochemical, morphological, immunological and physiological experiments proposed by the Soviet investigators are a continuation of joint U.S./U.S.S.R. rat experiments carried out on Cosmos-1887 and of experiments flown separately on the U.S.S.R. biosatellite Cosmos-1667 and on the U.S. space laboratory Spacelab-3.

Following the incorporation of the Soviet experiments additional foreign experiments were accepted as a result of the joint working group meeting with the French, Germans and Canadians. The SLS-1 BSP continued to grow with the inclusion of experiments from other U.S. sources including NIH, NASA, and various universities. Numerous unsolicited proposals which were received too late for incorporation prior to flight are under review for unclaimed tissues which were harvested and frozen by the ARC Project in anticipation of potential use.

## LIST OF CURRENTLY ACCEPTED **BSP EXPERIMENTS**

## SOVIET EXPERIMENTS

Experiment #1 Bone Biomechanics Investigators: A. V. Bakulin, Institute of Biomedical Problems, Moscow (IBP)

Experiment #2 Metabolic and Structural Changes in Bone and Systems Regulating Bone Growth and Metabolism.

Investigators: A. S. Kaplansky, I. A. Popova, G.N. Durnova, G.I. Plakhuta-Plakutina, E. I Alekseev, and T.E. Burkovskaya, Institute of Biomedical Problems, Moscow

Experiment #3 Osteogenesis - Tissue Factors of Regulation.

Investigators: V. S. Oganov, Institute of Biomedical Problems, Moscow

**Experiment #4 Lipid Peroxidation and Antioxidant** Defense System

Investigators: I.A. Popova, Institute of Biomedical Problems, Moscow

Experiment #5 Mechanisms of Formation of Gastric Hypersecretory Syndrome Investigators: K.V. Smirnov, Institute of Biomedical

Problems, Moscow

Experiment #6 Mechanisms of Changes in the Exocrine and Endocrine Functions of the Pancreas

Investigators: K.V. Smirnov, Institute of Biomedical Problems, Moscow

Experiment # 7 Study of the Digestive-Transportation Function of Small Intestine Investigators: K. V. Smirnov, Institute of Biomedical Problems, Moscow

Experiment #8 Effects of Space Flight Factors On the Functional Activities Of Immune Cells

Investigators: I. V. Konstaninova, Institute of Biomedical Problems, Moscow

Experiment #9 Primary Perceptive Structure of the Brain: Morphology and Histochemistry

Investigators: I.R. Krasnov, Institute of Biomedical Problems, Moscow

Experiment #10 Neuronal Morphology

Investigators: T. A Leontovich, and P.V. Belichenko, Brain Research Institute, Moscow

Experiment #11 Ultrastructure of the Brain Cortex Investigators: L.N. Dyachkova, Severtsev Institute of Evolutionary Morphology and Ecology of Animals, Moscow

Experiment #12 Cytochemistry of Brain Neurons Investigators: L.M Gershtein, Brain Research Institute,

Experiment #13 Contractile Properties of Skeletal

Investigators: V.S. Oganov, Institute of Biomedical Problems, Moscow

Experiment #14 Tissue Fluid - Electrolyte Composition Investigators: Yuri V. Natochin, Sechenov Institute of Evolutionary Physiology and Biochemistry, USSR Academy of Sciences, Lubov' V. Serova, Institute of Biomedical Problems

Experiment #15 Spinal Cord and Dorsal Root Ganglion Morphology and Histochemistry

Investigator: Igor B. Krasnov, Institute of Biomedical Problems; V.I. Drobyshev, I.V. Polyakov, Voronezh Medical Institute, Voronezh

Experiment #16 Histochemistry of Hypothalamus Investigator: Igor B. Krasnovm, Institute of Biomedical **Problems** 

Experiment #17 Morphology of Neurons of the Brain Cortex

Investigators: T. A Leontovich, M.A. Makhanov and P.V. Belichenko, Brain Research Institute, Moscow

## **CNES**

Catecholamines, Vasopressin, ANF and ANF Receptors in Rat Brain

Investigators: C. Gharib, University Physiologie de l'environnement. J. Gabrion and J.M. Pequignot, CNRS

Effect of Microgravity on the Relations Between Microbiological and Epithelial Tissue and Functions of the Gastrointestinal Tract.

Investigators: O. Szylit, I. Nugon-Baudon, C. Andrieux, Laboratoire Ecologie et Physiologie du Systeme Digestif. Dr. Ravisse, Unite d'histopathologie Institut Pasteur

#### **CSA**

ANF Changes in the Heart Investigator: A. J. Debol

#### DARA

Determination of ANF Receptors and of Particulate Guanylate Cyclase from Rats Flown in Weightlessness Investigators: R. Gerzer, Medizinsche Klinik Innenstadt der Universitat Ziemssentr 1

#### NIH

Effect of Space Flight on Cardiac Enzyme Activities Involved in Energy Metabolism
Investigators: R.S. Balaban and F.W. Heineman, National Heart, Lung, and Blood Institute

#### UCSD

Histologic Examination of Lung Tissue Investigators: J.B. West, O. Mathieu-Costello and A. Elliot, University of California, San Diego

#### NASA

Effects of Space Flight on Anterior Pituitary Receptors Investigators: R. Grindeland, NASA Ames Research Center, Moffett Field, California.

## **BSP** Implementation

The original BSP tissue harvest plan called for all of the tissues to be harvested and processed by an ARC Project Team in order to minimize the potential impact to SLS-1 Principal Investigator tissue collection. Support of this activity required extensive recruitment, training, and careful integration of a Project dissection team with the existing Pl teams. The Project dissection team of over 30 was integrated with the PI team which also number approximately 30 individuals (PI & their technicians). Initial training focused on individual tasks for each of the dissectors, which in the case of the Soviet and French experiments required the investigators to send dissection specialists to ARC to train the individuals collecting their specific tissues. Following individual task training the team was integrated into small groups and finally the groups into fully integrated team.

Since the facilities at the launch and the landing sites were very different, full-up dissection simulations were conducted to validate the critical timing of critical pre-dissection, dissection, and post-dissection activities. Both of the simulations identified numerous problems that could not have been anticipated and should be required of any future sharing program of this magnitude.

All of the flight and ground control dissections occurred as planned with only a few very minor errors, which is remarkable considering over 6000 tissues specimens were collected and processed by the team. A list of tissues taken is shown in Table 1 A, B, C, and D.

# PRELIMINARY REPORTS FROM BSP INVESTIGATIONS

#### Soviet Investigations

[Report received from Dr. Vyacheslav Korolkov]

Joint US/USSR investigations into the effects of microgravity and other space flight factors on mammals were initiated in 1975 on Kosmos-782 and continued through 1990 on Kosmos-936, -1129, -1514, -1667, 1887, and -2044. Joint US/USSR experiments made it possible to significantly expand the areas of research and to obtain a larger body of experimental results. The joint Kosmos studies helped to accumulate a great amount of experimental data concerning the physiological effects of microgravity and to gain an insight into the pathogenic mechanisms underlying various changes. The fruitful cooperation of US/USSR investigators aimed at studying space flight effects on the mammalian body received yet another impetus from development of joint studies to be flown on SLS-1 and SLS-2.

In 1991, USSR investigators took part, for the first time, in the realization of 17 joint rat experiments during the 9-day SLS-1 flight. The experiments included: 3 experiments to study bone morphology and biochemistry (experiments #1-3), 3 experiments to study biochemistry of the gastrointestinal system (experiments #5-7), 7 experiments to study cytochemistry and electron microscopy of the central nervous system, particularly brain vestibular structures (experiments #9-12 and #15-17), one experiment to study fluid and electrolyte metabolism (experiment #4), one experiment to study muscle contractility (experiment #13), and one experiment to study the immune system (experiment #8).

In the course of preparation of the flight experiments, US and USSR specialists met twice at NASA Ames Research Center where they practiced rat dissection. These rehearsals included training in the dissection, primary treatment, and conservation of biosamples to meet the requirements of the USSR PI's for all 17 experiments. After SLS-1 flight, US specialists performed rat dissections, weighed all organs and tissues, and prepared biosamples for these experiments at the recovery site. After that, the biosamples were shipped to NASA ARC where 6 USSR investigators continued biosample treatment, whenever it was necessary.

Altogether the USSR investigators were provided with biosamples from 25 rats from the following groups:

- (I) Basal controls
- (II) Flight rats sacrificed immediately after SLS-1 recovery
- (III) Controls [for II]
- (IV) Flight rats sacrificed at R+9 days
- (V) Controls [for IV]

Each of the groups included 5 rats. All biosamples were received by the institute of Biomedical Problems, USSR

Ministry of Health, Moscow in excellent condition. (The shipment requirements, including temperature requirements, were met making further laboratory analysis possible).

As of today [November 21, 1991], all the biosamples have been transferred to the different PI's who are actively working with them.

## French Investigations

[Report Received from Professor Claude Gharib, University Grande-Blanch, Lyon]

Ninety-eight frozen rat brains were received from NASA in September and rapidly dissected for the different purposes previously detailed, in brief, for checking:

- 1° AVP and ANP contents in hypothalamus and neurohypophysis.
- 2° Norepinephrine contents in catecholaminergic cell groups (A1, A2, A5, and A6).
- 3° ANP receptors in the choroid plexus.

## Purpose 1

Hypothalami were excised from whole brains. During this first step of our protocol, it was not possible to excise a well defined hypothalamic area from one group of animals (L+0 rats n° 6-15: RAHF control sacrificed at launch). [These] displayed a "smooth" aspect, suggesting a possible thawing before delivery. A second difficulty came from the fact that adenohypophysis halves were sent, instead of neurohypophysis (contact was taken with Mr. Meylor to receive the needed samples).

When samples will be received from NASA, they will be homogenesized, in the same time that the hypothalami and radioimmunoassays for AVP and ANP, in correlation with protein content determinations will be made.

#### Purpose 2

Brain stems were sectioned in 500 µm thick serial sections and A1, A2, A5, and A6 cell groups were punched in all the brains in which this part was intact. The brain stem was indeed sectioned in about two-thirds of the animals (5-7 in each group). Data are now obtained for the ten groups of rats.

As expected, in the control group sacrificed at launch (L+0), we observed a complete lack of norepinephrine that confirms a freezing-thawing problem for this group. In the other groups, we found an homogeneous distribution of the data in [a] given group. Contents appear, in a first approximation, quite regular in control groups, as shown by a comparison between R+0 (control animals, n° 36-45), DFPT R+0 (control animals, n° 111-120), and DFPT R+0 (flight animals, n° 101-120). It seems that slight differences can be determined in the flight animals (R+0 flight, n° 26-35), mainly at the level of A6 cell groups (Locus Coeruleus), involved in environmental changes. Preliminary results on SLS-1 experiments were compared with data obtained on catecholamine contents in brain stem nuclei of suspended rats maintained in individual plastic cages using a modified Morey's tail suspension model. These results seem to imply more stressful condition for animals sacrificed immediately after the flight.

TABLE 1A. SLS-1 Biospecimen Sharing Program AEM Tissues Summary List (tissues listed in alphabetical order)

Plight			(ti	ssues listed i	n alphab	etical order	)	
Acorta   yes	Tissue		Part Am	ount Ti	reatment	Recipient	Tissue Code	Comments
Monco	Adrenals*	ves		all	frozen	Project	ADRNLS	Left & rt. weighed together.
Product numb   Prod		•			frozen	German	AORTA	None
		•	_		frozen			None
Plasma	·	,		4.5 ml	frozen	Soviets	RBC-1	None
Pissma	шушшуш		_		frozen	Soviets	RBC-2	None
Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   Pick   None   Pick   Pick   Pick   Pick   None   Pick   Pick   Pick   Pick   None   Pick	Plasma		_		frozen	Soviets	PLS-2	None
Bones	1 mextua			0.1 ml	frozen	Soviets	PLS-3	None
Bones				0.1 ml	frozen	Soviets	PLS-4	None
Bones			_	0.1 ml	frozen	Soviets	PLS-5	None
Femur			_	rest	frozen	Project	PLS-1	None
Femur	Rones	no		_	_		_	None
		_	right	all w/o marrow	_		_	None
				frag. 1	fix	Soviets	FEM 1,2,3	In same vial as frag. 2 & 3.
Femure extract			_	-	fix	Soviets	FEM 1,2,3	In same vial as frag. 1 & 3.
Femure extract			_		fix	Soviets	FEM 1,2,3	In same vial as frag. 1 & 2.
Femure extract			_	_	Hd	Soviets	FEM 4A	In same vial as frag. 4B.
Femure extract			_	-	Hd	Soviets	FEM 4B	In same vial as frag. 4A.
Hcl	Femur extract		_		_	Soviets	HCL 4A	None
Humerus				Hcl		Soviets	HCL 4B	None
Tibia — right — — — — — — None    Seg 1A	(11)410220111114			Hcl	_	Soviets	HCL4C	Control
Tibia	Humenis	_	right	all w/o marrow	dried	Soviets	Z (RAT#)	None
		_				_	_	None
Seg 1B	110112		<del>-</del>	seg 1A	fix	Soviets	TIB 1A/V5	None
Seg 2   Frozen   Soviets   TIB 2   None			_	•	frozen	Soviets	TIB 1B	None
Seg 3   frozen   Soviets   TIB 3   None				_	frozen	Soviets	TIB 2	None
Seg 4   fix   Soviets   TIB 4   None				=	frozen	Soviets	ТТВ 3	None
Vertebral body — lumbar L5 fix Soviets L-VERT 5 None Vertebral body — lumbar L6 frozen Soviets L-VERT 6 None Brain no — — — — — None Brain tissue (frag. 4-1) — rt hemi 400 mg frozen Soviets BRAIN 4-1 None Hemisphere (frag. 12-1) — left all frozen Soviets HEMI-L None Hemisphere Vermis — right all frozen Soviets HM/VM None (frag. 9-3.) Hypothalamus — — all frozen Soviets HYPO None (frag. 16-1) Med obl/Pons varolii — left (9-4) 1/2 frozen Soviets MD-PN-L None sides (9-5)  Motor cortex (11-1) — rt hemi 2x4 mm fix (EM) Soviets MR-CX-R None Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets UNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets LNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets OF-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets LNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets OF-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets UNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets UNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets UNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None			_	•	fix	Soviets	T1B 4	None
Vertebral body — lumbar L5 fix Soviets L-VERT5 None Vertebral body — lumbar L6 frozen Soviets L-VERT6 None Brain no — — — — — None Brain tissue (frag. 4-1) — rt hemi 400 mg frozen Soviets BRAIN 4-1 None Hemisphere (frag. 12-1) — left all frozen Soviets HEMI-L None Hemisphere Vermis — right all frozen Soviets HM/VM None (frag. 9-3) Hypothalamus — — all frozen Soviets HYPO None (frag. 16-1) Med obl/Pons varolii — left (9-4) 1/2 frozen Soviets MD-PN-L None right (10-1) 1/2 fix (Golgi) Soviets MD-PN-R None sides (9-5)  Motor cortex (11-1) — rt hemi 2x4 mm fix (EM) Soviets MR-CX-R None Nodulus (frag. 9-1) — left medial all fix (EM) Soviets MNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets OF-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets UNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets OF-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None					frozen	Soviets	TIB 5	None
Vertebral body         —         lumbar         L6         frozen         Soviets         L-VERT 6         None           Brain         no         —         —         —         None           Brain tissue (frag. 4-1)         —         rt hemi         400 mg         frozen         Soviets         BRAIN 4-1         None           Hemisphere (frag. 12-1)         —         left         all         frozen         Soviets         HEMI-L         None           Hemisphere Vermis         —         right         all         frozen         Soviets         HM/VM         None           (frag. 9-3.)         —         —         all         frozen         Soviets         HM/VM         None           (frag. 16-1)         —         left (9-4)         1/2         frozen         Soviets         MD-PN-L         None           (frag. 16-1)         —         left (9-4)         1/2         frozen         Soviets         MD-PN-L         None           (frag. 16-1)         —         left (9-4)         1/2         frozen         Soviets         MD-PN-L         None           Modol/Pons varolii         —         left (9-4)         1/2         frozen         Soviets         MED-SL <t< td=""><td>Vertehral hody</td><td></td><td>lumbar</td><td></td><td>fix</td><td>Soviets</td><td>L-VERT 5</td><td>None</td></t<>	Vertehral hody		lumbar		fix	Soviets	L-VERT 5	None
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(frag 9-3.)  Hypothalamus — all frozen Soviets HYPO None  (frag. 16-1)  Med obl/Pons varolii — left (9-4) 1/2 frozen Soviets MD-PN-L None  right (10-1) 1/2 fix (Golgi) Soviets MD-PN-R None  slice, btwn slice fix (EM) Soviets MED-SL None  sides (9-5)  Motor cortex (11-1) — rt hemi 2x4 mm fix (EM) Soviets MR-CX-R None  Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None  Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None  Nodulus (frag. 9-2) — lateral all fix (EM) Soviets UNOD-L None  Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets UNOD-L None  Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None					_		HM/VM	None
Hypothalamus — — all frozen Soviets HYPO None  (frag. 16-1)  Med obl/Pons varolii — left (9-4) 1/2 frozen Soviets MD-PN-L None right (10-1) 1/2 fix (Golgi) Soviets MD-PN-R None slice, btwn slice fix (EM) Soviets MED-SL None sides (9-5)  Motor cortex (11-1) — rt hemi 2x4 mm fix (EM) Soviets MR-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets CF-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets CF-CX-R None			· <del>·o·</del> ··	_				
(frag. 16-1)  Med obl/Pons varolii — left (9-4) 1/2 frozen Soviets MD-PN-L None right (10-1) 1/2 fix (Golgi) Soviets MD-PN-R None slice, btwn slice fix (EM) Soviets MED-SL None sides (9-5)  Motor cortex (11-1) — rt hemi 2x4 mm fix (EM) Soviets MR-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets UNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None	-		_	all	frozen	Soviets	HYPO	None
right (10-1) 1/2 fix (Golgi) Soviets MD-PN-R None slice, btwn slice fix (EM) Soviets MED-SL None  Sides (9-5)  Motor cortex (11-1) — rt hemi 2x4 mm fix (EM) Soviets MR-CX-R None  Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None  Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None  Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None  Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None	• •							
slice, btwn slice fix (EM) Soviets MED-SL None sides (9-5)  Motor cortex (11-1) — rt hemi 2x4 mm fix (EM) Soviets MR-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None	Med obl/Pons varolii	_	left (9-4)	1/2	frozen	Soviets		
sides (9-5)  Motor cortex (11-1) — rt hemi 2x4 mm fix (EM) Soviets MR-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None			right (10-1)	1/2	fix (Golgi)	Soviets		
Motor cortex (11-1) — rt hemi 2x4 mm fix (EM) Soviets MR-CX-R None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None			slice, btwn	slice	fix (EM)	Soviets	MED-SL	None
Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None			sides (9-5)					
Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None	Motor cortex (11-1)	_	rt hemi	2x4 mm	fix (EM)	Soviets	MR-CX-R	None
Nodulus (frag. 9-1) — left, medial all fix (EM) Soviets MNOD-L None  Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None  Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None	Olfactory cortex (11-4)		rt hemi	2x4 mm	fix (EM)	Soviets	OF-CX-R	
Nodulus (frag. 9-2) — lateral all fix (EM) Soviets LNOD-L None  Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None	•	_	left, medial	all	fix (EM)			
Olfactory cortex (11-4) — rt hemi 2x4 mm fix (EM) Soviets OF-CX-R None	_	_	lateral	all	fix (EM)	Soviets	LNOD-L	
a company of the Comp	-		rt hemi	2x4 mm	fix (EM)	Soviets		
Solidioscisory (11-2)	Somatosensory (11-2)		rt hemi	2x4 mm	fix (EM)	Soviets	SS-CX-R	None
Cortex	•							
Somatosensory/ — rt. hemi 5x8 mm fix (Golgi) Soviets SM-CX-R None	Somatosensory/		rt. hemi	5x8 mm	fix (Golgi)	Soviets	SM-CX-R	None
Motor Cortex (17-2)	•							

\* Whole organ weight recorded. Note: The Soviets did not participate in the Delayed Flight Profile Test.

# TABLE 1A. (Continued) (tissues listed in alphabetical order)

		•		<b></b>			
Tissue	DFPT/ Flight	Part	Amount	Treatment	Recipient	Tissue Code	Comments
Visual cortex (17-1)	_	left hemi	5x8 mm	fix (Golgi)	Soviets	VS-CX-L	None
Visual cortex (11-3)	_	rt hemi	2x4 mm	fix (EM)	Soviets	VS-CX-R	None
Femur marrow	no	right	all	culture	Soviets	BN-MW	None
		ventride	300 mg slice	frozen	Soviets	HRT-Z	None
Heart*	yes	ventride	100 mg (apex	dried	Soviets	P (RAT#)	None
			of ventride)	•	n	ITOTA	NI
		atria	all	frozen	Project	HRT-A	None
Intestine	yes	duodenum	3 pieces	fix (1 vial)	Soviets	DUO-F	None
		duodenum	1 piece	frozen	Soviets	DUO-R	None
		jejunum	3 pieces	fix (1 vial)	Soviets	JEJ-F	None
		jejunum	1 piece	frozen	Soviets	JEJ-R	None
		ileum	3 pieces	fix (1 vial)	Soviets	ILE-F	None
		ileum	1 piece	frozen	Soviets	ILE-R	None
Kidney*	yes	left	100 mg	dried	Soviets	N (RAT#)	None
·		left	rest (300 mg)	frozen	Soviets	KID-Z	None
		right	all	frozen	Project	KID-R	None
Liver	yes	rt. lobe	100 mg	dried	Soviets	M (RAT#)	None
	•	rt. lobe	rest	frozen	Soviets	LIV-Z	None
		left lobe	portion	frozen	Project	LIV-L	None
Lung	yes		all	frozen	Project	LNG	None
Muscles	no	_	<del></del>	_		_	None
Brachialis	_	right	all	fix	Soviets	BRA-R	None
EDL	_	right	1/3, superficial	fix	Soviets	EDL-R	None
Gastrocnemius	_	right	lateral head	fix	Soviets	G-LAT-R	None
Gastrocnemius		right	medial head	fix	Soviets	G-MED-R	None
l lamstring		right	100mg	dried	Soviets	W (RAT#)	None
Rectus femoris	_	right	all	frozen	Soviets	RTF-R	None
Rectus femoris		left	ali	frozen	Soviets	RTF-L	None
Soleus	_	right	1/3, lateral	fix	Soviets	SOL-R	None
Triceps medialis	_	right	all	fix	Soviets	TRI-R	None
Vast. medialis		left	all	frozen	Soviets	VM-L	None
Vast. medialis		right	all	frozen	Soviets	VM-R	None
Pancreas	yes	upper 1/2	head	fixed	Soviets	PAN-H	None
	,	lower 1/2	tail	fix	Project	PAN-T	None
Pituitary	yes	′	all	fix	Soviets	PIT	None
Radius& Ulna	no	right	all	frozen	Soviets	R/U-R	None
Radius& Ulna	no	left	all	frozen	Soviets	R/U-L	None
Skin	yes	ventral	100 mg	dried	Soviets	Q (RAT#)	None
Spinal cord enlargements	no	cervical	upper 1/2	frozen	Soviets	CV-SC-E	None
opinar cord criming critical	110	œrvical	lower 1/2	frozen	Project	CV-SC-E2	None
		lumbar	upper 1/2	frozen	Project	LR-SC-E2	None
		lumbar	lower 1/2, .05 mm strip	fix (EM)	Soviets	L/DR	None
		lumbar	lower 1/2, rest	frozen	Soviets	LR-SC-E2	None
Spinal cord, ganglion,	no	rt. between	all	fix (EM)	Soviets	LSC/DR	In same vial as
dorsal rt.		L1-T12	<del></del>	(,			enlargement .05 m strip.
Spleen*	yes	_	1/3	culture	Soviets	SPLN	Not processed
6.			-11	<b>6</b>	C	cro) (	during DFPT.
Stomach	yes	-	all	frozen	Soviets	STOM	None
Testes*	yes	left	all	frozen	Project	TEST-L	None
		right	all	fixed	Project	TEST-R	None
Thymus*	yes		all	frozen	Project	THYM	None
Thyroid/Parathy.	yes	right lobe	all	fix (EM)	Soviets	TI IYR-R	None
		left lobe	all	fix	Soviets	THYR-L	None

<sup>\*</sup> Whole organ weight recorded.

Note: The Soviets did not participate in the Delayed Flight Profile Test.

TABLE 1B. SLS-1Principal Investigator AEM Tissues Summary List (tissues listed in alphabetical order)

110041	DFPT/ Flight	Part An	nount	Treatment	Recipient	Tissue Code	Comments
Blood, whole	yes	3 ml	_	_	Lange	_	Obtained from tail vein. Additional 250 µl samples were collected from R+0 (on L-3, L-2), R+ML (on L-4, L-3, R+0, R+1, R+4, R+8) animal group.
Bones	yes	_	<del></del>				None
Calvaria	_	_	all	frozen	Holton		R+ML flight gp only.
Femur		left	all w/o marrow	frozen	Holton	_	R+0/R+ML only
Humerus	_	left	all	frozen	Holton		R+0/R+ML only
Mandibular body	_	_	all	frozen	Holton		R+0/R+ML only
Maxilla and mandibular condyle	_	_	all	fix	Holton		R+0/R+ML only
Tibia		left	1/2 proximal	fix	Holton	_	R+0/R+ML only
11014		left	1/2 proximal	fix	Holton		R+0/R+ML only
		left	shaft	acetone	Holton	_	R+0/R+ML only
Vertebrae		_	L2	fix	Holton	_	None
		_	L3-L4	frozen	Holton	_	None
Diaphragm	yes		all				None
	,		1/2	fix	Riley		None
			1/2	frozen	Riley	_	None
Femur marrow	yes	left	all	slides/	Lange		None
2 0000000	,			culture			
Liver*	yes	left lobe	500 mg	counted	Lange		None
Muscles	yes		_	_	_		None
Adductor longus			all	frozen	Riley	_	None
Adductor longus	_	right	all	fix	Riley		None
Extensor digitorum long		left	2/3	frozen	Riley		None
Extensor digitorum long		right	2/3	frozen	Riley		None
Gastrocnemius	_	_	medial	frozen	Baldwin	_	None
Gastrocnemius		left	lateral	frozen	Baldwin		None
Plantaris		left	all	frozen	Baldwin		None
Plantaris		right	all	frozen	Riley	_	None
Soleus		left	2/3	frozen	Riley	_	None
Soleus	_	right	2/3	frozen	Riley	_	None
Tibialis anterior	_	left	all	frozen	Baldwin	_	None
Tibialis anterior	_	right	all	frozen	Baldwin	_	None
Vastus intermedialis	_	left	all	frozen	Baldwin	_	None
Vastus intermedialis	_	right	all	frozen	Baldwin	_	None
Vastus lateralis	_	left	all	frozen	Baldwin	_	None
Vastus lateralis	_	right	all	frozen	Baldwin	_	None
Thoracic and 1st lumbar vertebrae with rib cage	_	_	all	frozen	Holton	_	None
Spleen*	yes	2/3 whole	_		_	_	None
-t	<i>y</i>	<u> </u>	1/12	histology	Lange	_	None
		_	1/12	Lymph.	Lange	_	None
			1/2	Radioactive	Alfrey		None
			•	count	•		

<sup>\*</sup> Whole organ weight recorded.

TABLE 1C. SLS-1 Biospecimen Sharing Program RAHF Tissues Summary List (tissues listed in alphabetical order)

Tissue	DFPT/ Flight	Part	Amount	Treatment	Recipient	Tissue Code	Comments
Adrenals*	yes	_	all	frozen	Project	ADRNLS	Left & rt. weighed together.
Aorta	yes	_	all	frozen	German	AORTA	None
Blood, trunk	yes		_	<del></del>		_	None
Erythrocytes	,	_	4.5 ml	frozen	Project	RBC-3	None
_,,			4.5 ml	frozen	Project	RBC-4	None
Plasma		_	1.8 ml	frozen	Project	PLS-7	None
		_	لىر300	frozen	Project	PLS-8	None
			لىر300	frozen	Project	PLS-9	None
		_	rest	frozen	Project	PLS-6	None
Brain	yes	_	all	frozen	French	BRN-F	None
Cecum	yes	_	all	frozen	French	CECUM	None
Heart*	yes	_	300 mg (slice of ventricle)	frozen	NIH	HRT-VE	None
		_	100 mg (apex of ventride)	frozen	NIH	HRT-VX	None
			atria, all	frozen	Canada	HRT-A	None
Intestine	yes	duodenum	3 pieces	fix/ frozen	French	DUO-F	None
	_	duodenum	1 piece	frozen	French	DUO-R	None
		jejunum	3 pieces	fix/	French	JEJ-F	None
		,-,	- <b>F</b>	frozen			
		jejunum	1 piece	frozen	French	JEJ-R	None
		ileum	3 pieces	fix/	French	ILE-F	None
			-	frozen			
		ileum	1 piece	frozen	French	ILE-R	None
Kidney*	yes	right	all	frozen	Project	KID-R	None
,	,	left	all	frozen	Project	KID-Z	None
Liver*	yes	right lobe	all	frozen	German	ĽIV-R	None
	•	left lobe	portion	frozen	Project	LIV-L	None
Lung	yes	1/2	left	fix	West	LNG-W	None
8	•	1/2	right	frozen	German	LNGS	None
Pancreas	yes	upper 1/2	head	fixed	Project	PAN-H	None
	•	lower 1/2	tail	fix	Project	PAN-T	None
Pituitary	yes	all	_	_			None
ŕ	·	posterior	ali	frozen	Project	P-PIT	None
		anterior	right 1/2	frozen	French	R. Z-PTT	None
		anterior	left 1/2	frozen	Project	Z-PIT	None
Spleen*	yes		all	media	Sonnenfeld	SPLN	None
Stomach	yes	_	all	frozen	Project	STOM	None
Testes*	yes	left	all	frozen	Project	TEST-L	None
	•	right	all	fixed	Project	TEST-R	None
Thymus*	yes	_	all	frozen	Project	THYM	None

TABLE 1D. SLS-1 Principal Investigator RAHF Tissues Summary List (tissues listed in alphabetical order)

			1000000	iotoa iii aipiiai		<b></b> ,	
Tissue	DFPT/ Flight	Part	Amount	Treatment	Recipient	Tissue Code	Comments
Blood, whole	yes	3 ml	_	-	Lange	_	Obtained from tail vein. Additional 250 µl samples were collected from R+0 (on L-3, L-2), R+ML (on L-4, L-3, R+0, R+1, R+4, R+8) animal groups.
Bones	yes		<del></del>	_		_	None
Calvaria	<u>.</u>		all	frozen	Holton	-	R+ML flight & DPFT gps
Femur		right	1/2 distal	fix	Holton	_	None
		right	1/2 distal	fix	Holton	_	None
		right	shaft	acetone	Holton		None
Humerus	_	left	all	frozen	Holton	_	None
		right	1/2 distal	fix	Holton		None
		right	1/2 distal	fix	Holton		None
		right	shaft	acetone	Holton		None
Mandibular body	_	_	all	frozen	Holton	_	None
Maxilla and mandibular condyle		_	all	fix	Holton	_	None
Thoracic and 1st lumbar vertebrae with rib cage		_	all	frozen	Holton	_	None
Sacule, left		right	all	embedded	Ross		None
Sacule, right	_	right	all	embedded	Ross		None
Tibia		left	all	frozen	Holton		None
Tibia		right	1/2 proximal	fix (glut.)	Holton	_	None
		right	1/2 proximal	fix (formalin		_	In same vial as L2 Vertebrae.
		right	shaft	acetone	Holton	_	None
Utricle		left	all	embedded	Ross		None
Utricle		right	all	embedded	Ross	_	None
Vertebrae	_		1.2	frozen	Holton	_	In same vial as R. 1/2 proximal tibia.
Vertebrae			L3-L4	frozen	Holton	_	None
	1100		all	_	_		None
Diaphragm	yes		1/2	fix	Riley	_	First 5 animals
			1/2	frozen	Riley		First 5 animals
			all	frozen	Riley		Last 5 animals
Earness marrows	Voc	left	all	slides/	Lange		None
Femur marrow	yes			culture	-		
Liver*	yes	left lobe	500 mg	counted	Lange		None
Muscles	yes	_		_		_	None
Adductor longus (1st 5)	_	right	all	fix	Riley		None
Adductor longus (Next 5	5) —	<del>ri</del> ght	all	frozen	Riley		None
Adductor longus	_	left	all	frozen	Riley		None
Extensor digitorum longus (EDL) (1st 5)	_	right	1/3	fix	Riley		None
EDL (1st 5)		right	2/3	frozen	Riley		None
EDL (Next 5)		right	all	frozen	Riley	_	None
EDL		left	all	frozen	Riley	_	None
Gastrocnemius	_	right	lateral	frozen	Riley		None
Gastrocnemius	_	right	medial	frozen	Riley	_	None

<sup>\*</sup> Whole organ weight recorded

# TABLE 1D. (Continued) (tissues listed in alphabetical order)

Lissuc	DFPT/ Flight	Part	Amount	Ti	reatmen	Recipient	Tissue Code	Comments
Gastrocnemius	<del>_</del>	left	lateral	frozen	Baldwin		None	
Gastrocnemius		left	medial		frozen	Baldwin	_	None
Plantaris	_	right	all		frozen	Riley	_	None
Plantaris		left	all		frozen	Baldwin		5 rats only
Plantaris		left	all		frozen	Riley		5 rats only
Soleus (1st 5)	_	right	2/3		frozen	Riley		None
Soleus (1st 5)		right	1/3		fix	Riley	_	None
Soleus (Next 5)		right	all		frozen	Riley		None
Soleus	_	left	all		frozen	Riley	_	None
Tibialis anterior		right	all		frozen	Baldwin	_	None
Tibialis anterior		left	all		frozen	Baldwin	_	None
Vastus intermedialis		right	all		frozen	Baldwin	_	L+0 only
Vastus intermedialis	_	left	all		frozen	Baldwin		None
Vastus intermedialis (pyr.	) right	1/2	homogenizat	ion	Baldwin			R+0/R+ML only
Vastus lateralis	<i>—</i>	right	all		frozen	<b>Baldwin</b>	_	L+0 only
Vastus lateralis		left	1/2		frozen	Baldwin	_	None
Vastus lateralis	_	left	1/2 red		frozen	Baldwin		None
Vastus lateralis (pyruvate)	right	1/2 red	homogeniza	ion	Baldwin			R+0/R+ML only
Vastus lateralis (pyruvate)	_	1/2 whit	•		Baldwin	_	_	R+0/R+ML only
Vastus intermedialis (palr	•	1/2	homogeniza		Baldwin		_	R+0/R+ML only
Vastus lateralis (palmitate	_	1/2 red	homogeniza		Baldwin		_	R+0/R+ML only
Vastus lateralis (palmitate	_	1/2 whit	•		Baldwin	_		R+0/R+ML only
Otoconia (2 grids/rat)	yes	_	all		embedded	Ross	_	None
Spleen*	yes	2/3 who	le —				_	None
spicen	,		1/12		histology	Lange		None
		_	1/12		lymph.	Lange	_	None
		_	1/2		radioactive	Alfrey		None
					count	<u>=</u>		

## Purpose 3

Whole brains of animals in which it was not possible to study the catecholamine contents (see purpose 2) were sent at Montpellier. They are processed in 20  $\mu m$  thick sections for quantitative autoradiography of ANP receptors. Forebrains (still containing choroid plexus from lateral and third ventricles) of animals in which brain stems were sectioned for catecholamines study will be sent to Montpellier in mid-November. After their sectioning, quantitative analyses will be made in late '91 or early '92.

We are still waiting for the availability of other tissues for ANP (glomerular ANP receptors) and noradrenaline determinations (heart and kidneys).

[Report received from Dr. O. Szylit, Institut Pasteur, Paris]

Ninety-eight frozen rat intestines and cecal contents were received from NASA last September. They were shipped from Lyon and then stored to await analysis. The aim of our participation is to check whether the microgravity affects:

- Metabolic activities of intestinal flora determined with HPLC, spectrophotometry, and GC methods in cecal content.
- Cytochrome P450 and glutathione transferase in intestinal tissue. Glucuronosyl-transferase could be assayed if other results are encouraging.
- Histological and electronic microscopic observations of intestinal mucosal cells.

The rat samples obtained from experience SLS-1 were stored in 3 different deep-freezing units (-80°C) in our lab for security purpose. The technician hired for the analysis of those samples, directed by the 3 researchers involved in the program, has so far started treating 3 groups: RAHF (launch), RAHF flight and control (DFPT recovery).

- Cecal contents and walls were weighed, pH was controlled, and SCFA present in cecal contents have been already analyzed. Datas are being investigated. Bacterial activities (β-glucosidases, β-glucuronidases, nitro-reductases) are actually being assayed.
- From the December 15th to January 15th, the intestinal walls of those rats will be prepared for microsomes and some of Phase I and II enzymes assayed.

The next step will concern the RAHF flight and control recovery [groups] for all aspects.

## Dr. Gerzer's Investigation

[Report provided by John Meylor, LESC, NASA -Ames Research Center]

Aorta, lung, and liver samples from all RAHF groups were delivered to Dr. Rupert Gerzer in Munich, Germany, in September,1991. These samples were delivered on dry ice. Samples of aorta and liver have been confirmed in good condition. There is some uncertainty regarding the content of one container labelled "lung". The data records from the dissection are being investigated in order to resolve the discrepancy. Tissues are currently stored at -70°C at the PI's lab.

Following is a summary of Dr. Gerzer's proposed studies.

Previous results on rats flown on Spacelab have indicated that weightlessness induces changes in several cellular systems. Since the cellular responsiveness to stimuli depends at least in part on the subcellular distribution and properties of enzymes, these findings might indicate that the cellular responsiveness to hormones is altered in weightlessness and that determinations of plasma hormone levels alone do not necessarily allow conclusions on the state of a certain hormone system.

In order to find out about possible alterations of the responsiveness of the ANF/cyclic GMP systems in weightlessness, I will study the effects of weightlessness on the properties of ANF receptors and ANF-sensitive particulate guanylate cyclase in liver, aorta, and lung from rats flown for 9 days on the Spacelab Life Sciences 1 (SLS-1) mission. Determination of these reactions should also allow conclusions in man and thus help understand the process if the adaptation to weightlessness.

## **Determination of ANF Receptors**

The number and properties of ANF receptors will be determined in each studies tissue type using washed membranes of respective cell type. The binding characteristics - including binding, competition curves and affinities - will be determined by methods established in our laboratory. The computer program "ligand" will be used for calculating the binding and competition data. Competition curves will be done with ANF and at least two different analogues with different affinities for the  $R_{\rm 1}$  and  $R_{\rm 2}$  receptor.

The attained results will show whether the distribution and properties of ANF receptors are modified by weightlessness.

Determination of Particulate Guanylate Cyclase Activity

Unextracted membranes will also be used to determine the activity of particulate guanylate cyclase. The activity of this enzyme will be determined in the absence and presence of ANF (dose-response curve). Also, the influences of amiloride and of ATP will be determined in the presence of GTP\*Mn²+ or GTP\*Mg²+ as substrate, respectively. Amiloride and ATP sensitize the enzyme for activation by ANF and can thus show whether an alteration in the coupling mechanism has occurred.

## Separation of ANF Receptors

In a third step, ANF receptors are extracted from the membranes by TRITON-X-100 and extracted ANF receptors are subjected to SDS gel electrophoresis. This will separate  $R_1$  from  $R_2$ . In a further Western blot step including affinity labelling of the separated receptors, it will be possible to directly quantify the amounts of the respective receptor present.

## **NIH Investigations**

Report provided by John Meylor, LESC, NASA-Ames Research Center

Final tissue requirements are being defined for the investigation proposed by Dr. R.S. Balaban, Chief, Laboratory of Cardiac Energetics, NHLBI. The original tissue requirements (whole, 1 gram samples of LN<sub>2</sub> frozen rat hearts) could not be completely supported due to previous tissue

sharing commitments, however, the major objectives of the originally proposed study are still obtainable. Currently, the SLSPO project office is able to provide to Dr. Balaban LN, frozen sections of heart ventricles.

Following is a summary of the Dr. Balaban's proposed studies.

Energy for cardiac contraction is provided by adenosine triphosphate (ATP). ATP in the normal heart is produced mainly through oxidative phosphorylation occurring in the mitochondria. The capacity for oxidative phosphorylation can greatly influence the heart's performance and functional reserve. Thus, the cardiovascular deconditioning observed in space flight may be partially due to a down-regulation of the myocardial metabolic capacity during prolonged microgravity conditions. We are proposing to investigate this possibility by estimating the metabolic capacity of the heart using specific enzyme assays from frozen tissue samples.

The hearts collected from rats following space flight will not be useful for measurements of high energy phosphate compounds or for respiratory studies of intact myocytes or mitochondria due to the planned dissection and tissue processing. However, specific enzyme activities from tissue extracts can provide information on the metabolic capacity of the heart. These enzymes include: citrate synthase, providing an index of the matrix enzymes where the major reactions of the Krebs cycle are located; cytochrome aa<sub>3</sub>, to provide an indicator of the maximum oxidative capacity of the tissue; and creatine kinase, to reflect the ability of the hearts to transfer high energy phosphate intermediates between the sites of energy use (the myofibrils) and ATP production (at the mitochondria).

These three assays will provide information regarding the enzymatic apparatus responsible for maintaining the reducing equivalent supply to oxidative phosphorylation, the relative activity of the mitochondrial electron transport chain, and the cytosolic energy transport process. If space flight of microgravity alters one or more of these general facets of myocardial energy metabolism, it will help to direct future studies of cardiac deconditioning.

#### Dr. West's Investigation

[Report provided by John B. West, M.D., Ph.D., University of California at San Diego, La Jolla, CA 92093]

#### Introduction

The "Effects of Spaceflight on Lung Ultrastructure" was not one of the original SLS-1 animal experiments, but instead was added to the agenda only the year before launch. We have been given the opportunity to examine the lung tissue of the 19 rats flown in the RAHF during the 9-day SLS-1 mission. We also received lung tissue from four other groups of animals: basal controls; delayed-synchronous basal controls; flight controls; and delayed synchronous flight controls (Table 1). These controls were all maintained at 1-G conditions, and the delayed-synchronous controls were also exposed to similar environmental conditions as the flight animals.

Limited information is available regarding the effect of

microgravity on the lung. Several functional aspects of the respiratory system, such as alveolar size, alveolar ventilation, pulmonary blood flow, and respiratory mechanics have all been shown to be exquisitely sensitive to changes in gravity (West, 1977; Glaister, 1977). Microgravity exposure in man may cause a cephalad shift in body fluid. Pulmonary blood flow and alveolar ventilation becomes more uniform in microgravity (West, 1977; Michels, 1978). An increase in acceleration has been shown to accentuate the non-uniformity of pulmonary ventilation and blood flow (Glaister, 1977) as well as produce pulmonary interstitial edema (Weidner et al., 1981). Thus, exposure to changes in gravitational forces could potentially induce pathological changes in the lung related to abnormal lung fluid balance, altered pulmonary capillary hemodynamics and possible pulmonary hypertension. Our objective for this experiment is to examine the effects of microgravity exposure on lung ultrastructure and relate the changes in lung histology, if any, to alterations in lung physiology.

#### Methods

The lungs from each of the flight, delayed-synchronous, and basal control animals were removed from the thoracic cavity within 10 minutes of decapitation (Table 1). No precautionary measures were taken to ensure that aspiration of blood did not occur post-decapitation. One lung from each animal was immersed in glutaraldehyde (GA) fixative, (3% GA in 0.1M phosphate buffer total osmolarity of fixative: 560 mOsm; pH = 7.4 at room temperature), and then transported to our laboratory at 4°C.

First, a 3-4 mm thick tissue slab was cut perpendicular to the cranio-caudal axis just across the most caudal aspect of the hilum. Samples for electron microscopy were taken from the most ventral and dorsal aspects of the tissue slab. A piece of lung tissue (approx. 2mm x 2mm x 4mm) was removed from each region and further divided into 1mm x 1mm x 2mm cubes. The tissue samples were rinsed overnight in 0.1M phosphate buffer adjusted to 350 mOsm with NaCl. They were post-fixed for 2 hours in 1% solution of osmium tetroxide in 0.125M sodium cacodylate buffer adjusted to 350 mOsm with NaCl (total osmolarity: 400 mOsm, pH 7.4). They were dehydrated in increasing concentrations (70%-100%) of ethanol, rinsed in propylene oxide, and embedded in Araldite.

We are currently in the process of cutting sections, using an LKB Ultratome III, from two tissue blocks selected randomly from each lung site (dorsal/ventral). One micron thick sections are stained with 0.1% toluidine blue aqueous solution for examination by light microscopy. Ultrathin sections (50-70 nm) are contrasted with uranyl acetate and bismuth subnitrate (Riva, 1974) and examined with a Phillips 300 electron microscope.

The 1  $\mu$ m sections are systematically examined at magnifications of 400x and 1000x (oil immersion) for peribronchial cuffing of smaller pulmonary vessels, presence of alveolar edema, and general appearance of the pulmonary capillaries and lung parenchyma. The ultrastructure of the blood-gas barrier (capillary endothelium layer, interstitial space and epithelium layer) are examined by electron microscopy.

We also designed an experiment to examine the effects of

decapitation on lung ultrastructure. We decapitated two awake rats, as in the SLS-1 rat project, but fixed the lungs by vascular perfusion instead of immersion fixation. We perfused the lungs with normal saline for 3 minutes followed by 10 minutes of 2.5% GA in 0.1M phosphate buffer at a perfusion pressure of 20 cmH,O. In addition, we clamped the trachea before the chest cavity was opened so that the lung volume was maintained at functional residual capacity during fixation. This fixation procedure should allow us to more closely examine the ultrastructure of the blood-gas barrier, since both the capillary bed and the lung parenchyma will be more uniformly distended, unlike immersion fixed lungs, where capillaries remain filled with RBC or collapse and the lung tissue is not uniformly distended. We also decapitated two rats under halothane anesthesia. This allowed us to isolate and clamp the trachea before decapitation and assure that no blood would be aspirated. The lungs were then perfusion fixed in situ as described above. They were then removed from the chest and immersed in 2.5% GA fixative and stored at 4°C. The lung tissue was then processed as described for the SLS-1 rat tissue. We feel that both experiments are critical to assess the effect of decapitation per se on lung ultrastructure.

#### Results

The study is in progress and we anticipate the tissue analysis to be completed by June 1992. This study should provide information on the effect of microgravity on lung ultrastructure.

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## **TABLE 1. Animal Groups**

Basal Controls
Flight
Flight Controls
Delayed Synchronous Basal Controls
Delayed Synchronous Flight
Delayed Synchronous Controls

Time of	Sacrifice	e/# of Ani	<u>mals</u>
L-0	n=10		
R+0	n=10	R+ML	n=10
R+0	n=10	R+ML	n=10
"L-0"	n=10		
"R+0"	n=10	"R+ML"	" n=10
"R+0"	n=10	"R+ML"	" n=10

Note:

"ML" = Mission Length (Nominal 9 Days)

"L-0" = 22 Days Post R+0 (R+22)

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## APPENDIX 2

**Hardware Activities Post SLS-1** 

## **APPENDIX 2: Hardware Activities Post SLS-1**

## Research Animal Holding Facility (RAHF)

During preparation, integration, and flight of the Research Animal Holding Facility (RAHF) on Spacelab Life Sciences 1 (SLS-1), changes to the RAHF were identified for implementation prior to subsequent missions. These changes came from Ames Research Center (ARC) Engineering Change Orders (ECOs) and Kennedy Spaceflight Center (KSC) Problem Reports (PRs) with corresponding KSC Field Engineering Changes (FECs). These changes are listed in **Table 1**. Copies of documentation, representing these changes, are available from project files (contact David Mayer, (415-604-6804). (RAHF Project Office). Changes were reviewed and incorporated in the RAHF drawing set in order to show the "As-built/As integrated" configuration of the Rodent RAHF. While incorporating these changes the integration drawings were streamlined to better reflect integration flow. The second rodent RAHF was then brought into compliance with the upgraded drawing set.

In addition to the tasks and changes listed in the Table 1, the following tasks have also been performed:

- All RAHF drawings were formally transferred from LMSC document control to SLSPO document control.
- A stress analysis of RAHF was performed for SLS-2 which resulted in structural reinforcement of cooling water pump brackets, and cage module support brackets.
- To eliminate sources of corrosion which lead to drinking water manifold problems on SLS-1 two actions were taken. The lodinator was deleted from the water system, and nickel plated valve cores in the water manifold were replaced with solid brass units.
- Experiment Unique Feces Trays were designed for the SLS-2 bone experiment (Holton).
- General RAHF Refurbishment Tasks:
  - -Check/ Refurbish cooling water pump
  - -Change out 9V UEB Memory Backup Battery
  - -Clean Cage Module, ECS Screen, and SPAF Plenum
  - -Flush and Sterilize H<sub>2</sub>O lines

- -Check and replace condensate collector bag & check valve
- -Repair Quad 2 Lights

Table 1: Post SLS-1 Changes

Description of Problem/Task	Origin	FEC	Origin	Comment	
	Reference	No.	Org.		
GPTU Adapter spring-loaded locator wheels			Crew		
Fan pressure sensors do not indicate fan			LMSC	RAHF #2 worked OK in ground	N/A
operation, only other system pressure drops			<u> </u>	control	
Too short fasteners in Air Purification Installation 5701512 per KSC/QA requirements for 2 protruding threads	PR-SLS-1- MPE-STR-008	116	KSC		S
Rack mounted nutplate interference at	PR-SLS-1-R03-	125	KSC	See FEC 132	S
center post attach of drinking water panel	MPE-STR-020				S
Incorrect callout on Experiment Connector Bracket Installation	PR-T1-SLS-1- 1303A	126	KSC		
Wrong callout for fluid panel	PR-T1-SLS-1- 1303A	127	KSC		S
Some center post nutplates not needed	PR-T1-SLS-1- 1303A	128	KSC		S
Nutplates at rack corner posts will not accept flat head screws	PR-SLS-1-R03- MPE-STR-012	129	KSC		S
Drinking water system interferes with rack diagonal strut	PR-SLS-1-R03- MPE-STR-020	131	KSC	Incorporate installation sequence on rack drawing	S
Drinking water system interferes with rack diagonal strut; relocate and make new nutplates	PR-SLS-1-R03- MPE-STR-020	132	KSC	Investigate relevance of related FECs 125,131, and 163.     Modify as needed; may need RAHF Office support	S
LMSC 5817713 specifies left side mounted	PR-SLS-1-R03- MPE-STR-017	134	KSC		S
tank but tank mounted on right Air purification installation interferences	PR-SLS-1-R03- MPE-STR-021	135	KSC		S
Nuts on Air Pure system interfere	PR-SLS-1-R03- MPE-STR-021	136	KSC		S
Drawings do not specify two thread protrusion	PR-SLS-1-R03- MPE-STR-020	137	KSC	General note on drawing	S
Multiple problems on coolant pump installation drawing	PR-SLS-1-R03- MPE-STR-023	141	KSC		S
Shimming on FEC #131 now requires new shims in Air Pure Inst!	PR-SLS-1-R03- MPE-STR-021	142	KSC		S
Water tank bracket will not fit in attach brackets	PR-SLS-1-R03- MPE-STR-035	146	KSC		S
Drinking water tank interferes with rack	PR-SLS-1-R03- MPE-STR-035	148	KSC		S
Avionics hose clamp replaced with MPE item	PR-T1-SLS-1- 1303A	149	KSC		S
Cannot adequately secure bleed air hoses	PR-SLS-1-R03- MPE-STR-029	150	KSC		S
Water tank bracket interferes with nutclip	PR-SLS-1-R03- MPE-STR-032	151	KSC		S
Charcoal filter angle interferes with UEB parts	PR-SLS-1-R03- MPE-STR-037	159	KSC		S
Cable clamps on water manifold 5701513 cannot be used	PR-SLS-1-R03- MPE-STR-038	163	KSC	See FEC 132	S
Air Purification System Installation fasteners interfere with mounting brackets		164	KSC		S

		105	1400		S
Modify cable routing to suit KSC preference	PR-T1-SLS-1- 1303A	165	KSC		
Difficult installation of Cage Module	PR-T1-SLS-1- 1303A	166	KSC		S
Incorrect part number on Experiment Connector Bracket	PR-SLS-1-R03- MPE-STR-022	170	KSC		S
Incorrect part number on Rack Top Assembly (CRAN-381)	PR-T1-SLS-1- 1303A	171	KSC		S
Drinking Water Tank Installation drawing	PR-T1-SLS-1- 1303A	172	KSC		S
errors Water Tank Installation problems; self locking fasteners limited to single insertion	PR-T1-SLS-1- 1303A	173	KSC		S
Tube clamp induces stress in tubing and is not needed on rack bulkhead feed thru for adequate support	PR-T1-SLS-1- 1303A	175	KSC		S
Module top insulation panel 5701823 attach holes do not align to module	PR-SLS-1-R03- MPE-STR-013	181	KSC		S
U bracket attach at UEB mount to left rear rack post misaligned with right post	PR-SLS-1-R03- MPE-STR-037	185	KSC		S
Water tank support interference with staged rack nutplate	PR-SLS-1-R03- MPE-STR-044	186	KSC		S
Primary SPAF front panel interferes with rack bolt	PR-SLS-1-R03- MPE-STR-068	187	KSC		S
Primary SPAF support rail interferes with staged rack nutclip	PR-SLS-1-R03- MPE-STR-075	194	KSC		S
No KSC spares available for UEB locking nut PSI-4791-3 (structure ground lug)	PR-SLS-1-R03- MPE-ELE-008	198	KSC		S
Aux SPAF Support Assy interferes with staged nutclip and misaligned with rack holes	PR-SLS-1-R03- MPE-STR-075	203	KSC		S
Coolant pump installation interferes with -002 Locker	PR-SLS-1-R03- MPE-STR-059	206	KSC		S
Misc problems with rack insulation installation	PR-T1-SLS-1- 1303A	207	KSC		S
ECS insulation attach screw too short	T1-SLS-1- 1303A	208	KSC		S
Misc experiment connector bracket problems	PR-SLS-1-R03- MPE-STR-014	209	KSC		S
Insufficient support to water separator delivery hose	PR-SLS-1-R03- MPE-STR-070	210	KSC		S
LEB metric attach screws not provided to KSC	PR-SLS-1-R03- MPE-STR-065	211	KSC		S
Module structural Beta tape unravels; Unacceptable adhesive		212	KSC	Add Mystic 7000 tape to assembly drawing	
Screw interference on experiment connector bracket installation, AD800-895D-M104	PR-SLS-1-R03- MPE-STR-013	213	KSC		S
Insufficient cable clamps	T1-SLS-1- 1303A	219	KSC		S
SPAF support installation interference with	T1-SLS-1- 1303A	220	KSC		S
rack nutclip Attach screws may mar GPTU attach	T1-SLS-1- 1303A	221	KSC	Add washers to installation drawing	S
brackets MS27039C1-18 screw not available for	PR-T1-SLS-1- 1303A	223	KSC		S
5701509 cage module installation Condensate flex line too short	PR-SLS-1-R03- MPE-STR-066	228	KSC	Modify length per FEC	S
UEB attach screw too short; must share hole	PR-SLS-1-R03- MPE-STR-061	229	KSC		S
location with 8S locker bracket Sharp edges in rack may damage coolant hose insulation	PR-SLS-1-R03- MPE-STR-072	230	KSC		S

Primary SPAF requires Type 3 clip, normally pre staged, to attach to nutclip during installation  The new 1/2 inch under floor water coolant lines do not connect to 5/8 inch RAHF fittings  Coolant line Beta tape unravels  Cable ties not adequate to secure coolant lines  Coolant lines excessively long in double rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2  KSC likes flat washers on both sides of nut/bolt fasteners  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic coupling on startup  PR-SLS-1-R03- MPE-STR-081  PR-SLS-1-R03- 285  KSC   KSC   SC   SC   SC   SC   SC   SC
installation The new 1/2 inch under floor water coolant lines do not connect to 5/8 inch RAHF fittings MPE-STR-081 Coolant line Beta tape unravels  PR-SLS-1-R03- MPE-STR-081 Coolant lines excessively long in double rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2 KSC likes flat washers on both sides of nut/bolt fasteners Cables not secured at enough points (usual KSC rule to have clamp 15 inches min) Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage TEU discrete reads +12 V unless external load attached (open collector) Water separator motor breaks magnetic  PR-SLS-1-R03- MPE-STR-088 RSC   SSC
The new 1/2 inch under floor water coolant lines do not connect to 5/8 inch RAHF fittings    Coolant line Beta tape unravels    Cable ties not adequate to secure coolant lines excessively long in double rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2  KSC likes flat washers on both sides of nut/bolt fasteners    Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)    Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage    TEU discrete reads +12 V unless external load attached (open collector)    Water separator motor breaks magnetic    PR-SLS-1-R03- MPE-STR-081    FR-SLS-1-R03- MPE-STR-088    SC     KSC     LMSC     1. Add reducers per FEC    2. SLS-2 to use all 3/8" line    S     KSC     S     KSC
Innes do not connect to 5/8 inch RAHF fittings   MPE-STR-081   2. SLS-2 to use all 3/8" line
Coolant line Beta tape unravels  PR-SLS-1-R03-MPE-STR-085  Cable ties not adequate to secure coolant lines  Coolant lines excessively long in double rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2  KSC likes flat washers on both sides of nut/bolt fasteners  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  PR-SLS-1-R03-MPE-SLS-1-R03-MSC KSC MSC MSC MPE-ELE-014  N/A KSC Cured by T-0 multiplexer  N/A LMSC RAHFs #1 and #2 worked first time; N/A  LMSC RAHFs #1 and #2 worked first time; N/A
Cable ties not adequate to secure coolant lines  Coolant lines excessively long in double rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2  KSC likes flat washers on both sides of nut/bolt fasteners  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  MPE-STR-085  PR-SLS-1-R03- AD  FO 5701531- (285)  LMSC  S LMSC  S KSC  MSC  MSC  MSC  MSC  MPE-SLS-1-R03- MPE-ELE-014  Buckendahl  N/A  Crew  1. See SPAF variable blower  N/A  KSC  Cured by T-0 multiplexer  N/A  LMSC  RAHFs #1 and #2 worked first time; N/A
Cable ties not adequate to secure coolant lines  Coolant lines excessively long in double rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2  KSC likes flat washers on both sides of nut/bolt fasteners  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  PR-SLS-1-R03- AD  EO 5701531- (285)  EMSC  FR-T1-SLS-1- 320  KSC  MPE-T1-SLS-1- 320  KSC  MSC  S  KSC  KSC  KSC  S  KSC  KSC  KSC  S  KSC  N/A  KSC  KSC  KSC  KSC  KSC  KSC  KSC  KS
lines MPE-STR-088  Coolant lines excessively long in double rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2  KSC likes flat washers on both sides of nut/bolt fasteners  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  MPE-STR-088  EO 5701531- (285)  LMSC S  KSC S  KSC S  KSC S  KSC S  MPE-T1-SLS-1- 320  KSC S  KSC S  KSC S  MPE-ELE-014  N/A Crew 1. See SPAF variable blower  Cured by T-0 multiplexer N//  N/A LMSC RAHFs #1 and #2 worked first time; N//
Coolant lines excessively long in double rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2  KSC likes flat washers on both sides of nut/bolt fasteners  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  EO 5701531- AD  EN SC  KSC  KSC  KSC  KSC  KSC  KSC  KSC
rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2  KSC likes flat washers on both sides of nut/bolt fasteners  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  AD  PR-T1-SLS-1-320 KSC S  KSC KSC S  KSC KSC S  MPE-ELE-014  N/A Crew 1. See SPAF variable blower  1. See SPAF variable blower  N/A KSC Cured by T-0 multiplexer N/A  N/A LMSC RAHFs #1 and #2 worked first time; N/A
rack; drawing now allows shorter hoses; not incorporated on Rodent RAHF#1 or #2  KSC likes flat washers on both sides of nut/bolt fasteners  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  AD  PR-T1-SLS-1-320 KSC S  KSC KSC S  KSC KSC S  MPE-ELE-014  N/A Crew 1. See SPAF variable blower  1. See SPAF variable blower  N/A KSC Cured by T-0 multiplexer N/A  N/A LMSC RAHFs #1 and #2 worked first time; N/A
KSC likes flat washers on both sides of nut/bolt fasteners  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  PR-T1-SLS-1- 320 KSC SC
nut/bolt fasteners 1303A
nut/bolt fasteners 1303A  Cables not secured at enough points (usual KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  1303A  PR-SLS-1-R03- MPE-ELE-014  Buckendahl  N/A  Crew  1. See SPAF variable blower  1. See SPAF variable blower  Cured by T-0 multiplexer  N/A  LMSC  RAHFs #1 and #2 worked first time; N/A
KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  MPE-ELE-014  Buckendahl  N/A  Crew  1. See SPAF variable blower  N/A  KSC  Cured by T-0 multiplexer  N/A  LMSC  RAHFs #1 and #2 worked first time; N/A
KSC rule to have clamp 15 inches min)  Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  MPE-ELE-014  Buckendahl  N/A  Crew  1. See SPAF variable blower  N/A  KSC  Cured by T-0 multiplexer  N/A  LMSC  RAHFs #1 and #2 worked first time; N/A
Crew observation that inserting a waste tray cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  Buckendahl  N/A  Crew  1. See SPAF variable blower  N/A  KSC  Cured by T-0 multiplexer  N/A  LMSC  RAHFs #1 and #2 worked first time; N/A
cover with SPAF on causes the debris to blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  N/A LMSC RAHFs #1 and #2 worked first time; N/A
blow to the rear of the cage  TEU discrete reads +12 V unless external load attached (open collector)  Water separator motor breaks magnetic  N/A LMSC RAHFs #1 and #2 worked first time; N/A
TEU discrete reads +12 V unless external load attached (open collector)  N/A KSC Cured by T-0 multiplexer N/A load attached (open collector)  N/A LMSC RAHFs #1 and #2 worked first time; N/A
load attached (open collector)  Water separator motor breaks magnetic  N/A  LMSC  RAHFs #1 and #2 worked first time; N/A
Water separator motor breaks magnetic N/A LMSC RAHFs #1 and #2 worked first time; N/A
RAHF
requirement can be met using ±12 VDC
supply.
TEU fans do not cycle with heater NCR #I-2361 N/A ARC Rework to existing EOs R
operations; over stressed transistor and
clamped logic drive
Primary SPAF louvre interferes with N/A KSC Rework louvre R
Spacelab liftup floor panel by 1 inch
Increase UEB potentiometer adjustment
span by changing resistor values; not 2. Rework as needed
incorporated on Rodent RAHF#1
Original vendor P/N on RTV used to seal
module fan leaks changed to new P/N for drawings
equivalent material
Rodent status on-pad not known during 24 N/A N/A ARC 1. Develop 2 wire multiplexer for T- R
hour launch scrub 0 transmission of all RAHF data
2. Develop 2 wire reset for T-0 for
concurrent 24 water alarms and 1
TEU over-temp alarm
Drinking water tank pressure sensor W. Hinds N/A ARC 1. Calibrate sensor and integral R
resolution too gross for use as backup to signal conditioner
aliquot calibrations  2. Increase RAU span to 12 psi =
-5VDC and 60 psi = +5VDC
3. Prepare appropriate change
request for ECAS and SEI
software update.
Cage air flow balancer (pseudo cage)  Crew debrief  N/A  Crew  Develop adjustable module  R
cumbersome to use during repeated SLS-2 mounted air inlet devices that will
cage extractions  (a) admit light when open, (b) act
j j jagair hirning vande to minimiza i
as air turning vanes to minimize
rear cage turbulence, and (c) close
rear cage turbulence, and (c) close off cage air flow when closed
Urine leaks around front cage windows  Buckendahl  N/A  ARC  Add RTV sealant to perimeter quad R
Urine leaks around front cage windows  Buckendahl  N/A  ARC  Add RTV sealant to perimeter quad ring seal on Lexan window
Urine leaks around front cage windows  Buckendahl  N/A  ARC  Add RTV sealant to perimeter quad R

	<del></del>	1 4 1 / 4	Tuoo	To the cost	T D
Drinking water manifold calibration poor because of air entrapped	Jahns	N/A	KSC	Develop suitable GSE to vacuum evacuate at the cage module quick disconnect. Item must provide all services and require only a 110 VAC external power source.	
GN2 pressure in water tank lost by diffusion through bladder during 8 month hiatus	N/A	N/A	KSC	Extend tank GN2 fill to rack front panel     Develop suitable GSE for refill	R
Drinking water solenoids sticking closed; suspect corrosion buildup on plunger	No PR Identified	N/A	KSC	Inspect all manifolds for sleeve/plunger dimensions     Rework as needed.	R
RAU clock input circuit for PCM data had improper 1984 fix reducing signal level by one half producing marginal performance	Buckendahl	N/A	ARC	Modify as needed	R
Thermistors may have drifted with time. No adjustable amplifier available to compensate.	Buckendahl	N/A	ARC	Perform lab ambient verification test and record output volts to RAU.     Prepare, if needed, request for ECAS and SEI software update.	R
Update RAHF drawings to include outstanding hardware assembly FECs as required (does not include installation FECs)	Hogan	N/A	ARC	Modify as required	R
Wrong screw installed in 5701578-501 assembly on Rodent #2; interferes with rack	NCR #I-2427	N/A	ARC		S
Rodent Cage door not bonded to structure; violates 1 ohm requirement for anti-static buildup	Waiver ARC- SLS1-037	N/A	ARC	Letter from MMO	S
SPAF flat cover not reversible on Rodent #1	NCR #H-229	N/A	ARC	Inspect if true     Mark "UP" on cover if true	S
End of 5701829-1 bleed air hose crushed	NCR #I-2360, I- 588	N/A	ARC	Caution note on rack installation	S
SLS-1 Cage S/N 009 and 012 with PCDT not configured with correct floor grid		N/A	ARC	Rework as needed	S
KSC Test Personnel prefer drinking water sample "stingers" for routine activities	N/A	N/A	KSC	Develop replacement GSE     Determine quantity needed	S
Monstrous pain to verify water tank gas pressure using the fill cart gage	N/A	N/A	KSC	Resolved by new water fill GSE	S
Add support clamp to Rodent drinking water lines; not incorporated on Rodent RAHF #1	EO 5701512- AK	N/A	LMSC		S
Coolant pump Z mounting bracket has low stress margin; change from 5701669-7 to -9; not known if incorporated on Rodent RAHF#2 or Primate	EO 5701521- AA	N/A	LMSC	Inspect     Rework as needed	S
Module mounted thermistor guards provide limited air circulation; add two vent holes; not known if incorporated on Rodent #1	EO 5818226-B	N/A	LMSC	Ain't broke; don't fix it	S
Formal fracture disposition required	NHB 8070.1	N/A	ARC	Prepare plan     Perform needed inspections     Replace affected assembly bolts	S
1 rodent in each of flight and ground control lost weight; opinion that food bar "jumped" and snapped rodent nose	Dalton	N/A	ARC	Questionable Priority; resolution by RPH/BPD 1. Longer period in training cages 2. Redesign food bar ramps to better break off chisel points	S
Wear and tear on flight cables	Buckendahl	N/A	ARC	Inspect all cables for damage     Test all cables for conformance to requirements on drawing	S
Humidity sensors drift with time	Buckendahl	N/A	ARC	Obtain Manufacturer's     saturated salt calibration kits     Calibrate	S

Rodent Cage attach screws bind on release latch; attributed to lot length variations;		N/A	ARC	Rework as needed	Т
incorporate LMSC EO that shortens screws as needed					
Paint splatter inside SPAF cover latches	NCR # G-235 (closed)	N/A	ARC	Inspect	T
Quad #1 temp data erratic; cause found to be #6 screw backed out on TB-1 causing open amplifier input	PR-SLS-1-EXP- RAHF-015	N/A	KSC	Inspect     Rework as needed	T
Humidity sensor #1 data erratic; sensor shield grounding intermittent	PR-SLS-1-EXP- RAHF-012	N/A	KSC	Rework to existing EO	T
Remove cadmium plated screws from UEB; not known if incorporated on Rodent RAHF#1	EO 5701521- AC	N/A	LMSC	Inspect     Rework as needed	T
Possible to destroy the dynamic balance of the 3000 rpm water separator if cage assembly rotated on motor shaft after refurbishment; scribe matching marks on parts	EO 5701567-T	N/A	LMSC	Replace and run-in new bearings.     Dynamically rebalance cage	Т
Condensate collector velcro adhesive releases after repeated actuations; add rivet hold down; not positively known if incorporated on Rodent #1	EO 5701581-H	N/A	LMSC	Inspect     Rework as needed	T
Water pressure regulator mounting nut loosens easily; add Loctite; not known if incorporated on Rodent #1	EO 5701786-N	N/A	LMSC	Inspect     Rework as needed	ĪŤ
Replace cadmium plated hardware in SPAF; not known if incorporated on Rodent #1	EO 5813106-J	N/A	LMSC	Inspect     Rework as needed	Ť
Cage AMS 3195 die-cut gaskets may have suffered during extensive handling	Houston	N/A	LMSC	Inspect and replace as needed     Test all for total front face     leakage using existing GSE	T
Flight activity monitors not functional	Jahns	N/A	ARC	Test for failed units     Replace with spares tested and modified with apertures	Τ
Screws on terminal blocks may back out during vibration	Lobenberg	N/A	LMSC	Rework to EO	T
Brush life on water separator fan limited to 700 hours. Fan cage bearings may also be contaminated from condensate.	Buckendahl	N/A	ARC	Send fans to manufacturer for refurbishment     Assure adequate spares or refurbishment plan to support EVT	Т
Charcoal canister saturated with animal odor	Buckendahl	N/A	ARC	Repack with acid treated BD charcoal	T
Bleed air outlet and inlet HEPA filters likely loaded with particulates	Buckendahi	N/A	ARC	Inspect for particulates     Carefully vacuum debris and perform low pressure air flush	T
SLS-1 unit has many accumulated test and flight hours; DC brushless motor bearings require inspection!!! Which RAHF??????	Buckendahi	N/A	ARC	Inspect all units (2 bleed air, 4 circulation, and 2 TEU) for bearing noise. WARNING! Pam Motors no longer made and no alternate known	T
Primary SPAF Installation 5813111 requires addition of MPE Attach Clip Type 3	PR-SLS-1-R03- MPE-STR-067	Uknwn	KSC		S

# APPENDIX 3

**Summary Food and Water-Consumption Data** 

SLS-1 AEM Food and Water Consumption - Flight and DFPT

	Flight Test				DFPT			
Group:	GC R+0	GC R+ML	Flight R+0	Flight R+ML	GC R+0	GC R+ML	Flight R+0	Flight R+ML
, de la composition della comp	AEM 001	AEM 004	AEM 002	AEM 003	AEM 001	AEM 004	AEM 002	AEM 003
Bat ID # 1	10	15	16	13	21	06	14	40
6	42	47	49	45	33	95	23	55
ı co	71	79	80	74	44	116	26	65
4	106	112	113	109	61	132	29	88
2	139	145	146	142	72	154	163	135
Preflight Unconsumed Food Wts	med Food Wts							
Right Cage	709.2	710.2	712.1	713.5	708.7	732.4	713.9	731.6
Left Cage	709.1	711	710	712.7	714.5	734.9	741.7	735.2
Right Water Box	537.4	542.3	537.6	536	534.1	551.1	555	556.4
Left Water Box	536.8	533.7	537.2	537.6	533.3	551.4	557.5	556.9
TOTAL	2492.5	2497.2	2496.9	2499.8	2490.6	2569.8	2568.1	2580.1
Postflight Unconsumed Food Wts	umed Food W	ts.						
Right Cage	151.2	465.2	513.7	349	149.82	190.68	231.54	304.18
Left Cage	144.5	290.8	347.3	425	190.68	508.48	204.3	177.06
Right Water Box	436.1	124.6	111.3	113.4	108.96	131.66	413.14	376.82
l eft Water Box	355.6	219.9	109	173	435.84	136.2	172.52	199.76
TOTAL	1087.4	1100.5	1081.3	1060.4	885.3	967.02	1021.5	1057.82
AFood	1405.1	1396.7	1415.6	1439.4	1605.3	1602.78	1546.6	1522.28
AFood/Dav	134.1	133.3	135.1	137.3	153.2	152.9	147.6	145.3
ΔFd/Rt/Dy	26.8	26.7	27.0	27.5	30.6	30.6	29.5	29.1
Droffight Water	1255	1280	1275	1270	1210	1285	1205	1220
Dofill #1	069	570	1775.7	1775.7	940	875	890	905
Refill #2	685	525			1040	770	850	006
Refill #3	635	395						:
Postflight water	695.8	663.9	774.5	1069.7	440	390	410	565
TOTAL AWater	2569.2	2106.1	2276.2	1976	2750	2540	2535	2460
∆ WVRVDv	48.9	40.1	43.4	37.6	52.4	48.4	48.3	46.9
			40.5	all flight		47.5	all ground	

SLS-1 RAHF Food Bar Consumption During Flight and DFPT

# Cage S/N 1 20 20 20 2 2 2 2 4 4 4 4 4 4 24 24 24 25 7									
14 18 18 28 38 38 38 44 48 58 68 68 68 68	st Feeder	New FB wt	Old FB wt	Stow FB S/N	New FB wt	Old FB wt	ΔFB wts	FB wt/rt/dy	Δ from aver
18 2A 3A 3A 4A 4A 4A 4B 5A 5A 6A 6A 7A	က	340.0	0.06	2	345.0	307.5	287.5	27.4	1.0
2A 2B 3A 3A 4A 4B 5A 5A 6A 6A 7A	3	345.0	40.0	2	345.0	308.4	341.6	32.5	1.4
28 3A 4A 4B 4B 5A 5A 6A 6A 7A	2	340.0		30	355.0	345.7	349.3	33.3	
3A 3B 4A 4B 5A 5A 6A 6A 6A	2	345.0		30	350.0	348.4	346.6	33.0	
38 4A 4B 4B 5A 5B 6A 6A 7A	13	340.0	65.0	6	345.0	312.8	307.2	29.3	-0.9
4A 4B 5A 5B 6A 6B 7A	13	335.0	70.0	6	345.0	310.4	299.6	28.5	٠ 1.0
5A 5B 6A 6B 7A	4	345.0	58.4	17	355.0	319.6	322	30.7	-2.3
5A 5B 6A 6B 7A	4	345.0	90.5	17	355.0	325.1	284.4	27.1	1.3
58 6A 6B 7A	24	345.0	49.7	21	350.0	322.8	322.5	30.7	-2.3
6A 6B 7A	24	345.0	114.1	21	350.0	325.0	255.9	24.4	4.0
6B 7A	5	340.0	0.96	16	350.0	330.1	263.9	25.1	3.3
7A	5	340.0	340.0	16	345.0	345.2	-0.2	0.0	
	7	340.0	79.2	28	345.0	313.5	292.3	27.8	9.0
78	7	350.0	72.0	28	355.0	324.5	308.5	29.4	-1.0
8A	ω	345.0	105.4	18	355.0	324.8	269.8	25.7	2.7
8B	8	350.0	86.4	18	355.0	321.4	297.2	28.3	0.1
9 <b>A</b>	6	350.0		20	350.0	333.7			
7 9B	6	345.0		20	355.0	324.0			
156 10A 10	10	340.0	105.7	29	350.0	324.7	259.6	24.7	3.7
127 10B 10	10	345.0	74.7	29	350.0	319.5	300.8	28.6	-0.2
11A	23	355.0	48.4	25	350.0	316.7	339.9	32.4	-4.0
118	23	345.0	99.2	25	350.0	317.2	278.6	26.5	1.9
12A	12	345.0	67.9	19	355.0	318.0	314.1	29.9	-1.5
162 12B 12	12	340.0	52.9	19	350.0	318.4	318.7	30.4	-2.0
TOTALS		8255.0	1805.5		8410.0	7757.4	6359.8	605.7	
Aver.		344.0	77.1		350.4	319.0	298.1	28.4	
SD		4.4	21.7		3.9	6.3	25.4	2.4	
Notes: Feeders from PCDT cages 2 and 9 were placed in cages 1	PCDT cage	s 2 and 9 w	vere placed i	n cages 1 and 2	and 2 during feeder changeout.	er changeout			
It is not clear which feed	r which feed	der went in v	er went in which cage.						

SLS-1 RAHF Food Bar Consumption During Flight and DFPT

				The second of the second						
Rat ID   Cade #	# Cade S/N	1st Feeder	New FB wt	Old FB wt	Stow FB S/N	New FB wt	Old FB wt	ΔFB wts	FB wt/rt/dy	Δ from aver.
ر 1	22,22	:		77.2	6	350.0	313.3	309.56	29.5	-1.2
3 1B	21	8	350.0	8.06	6	355.0	313.3	300.94	28.7	-0.4
<u> </u>	6	10	345.0				336.0	9.04	6.0	
-	ō	10	350.0				340.5	9.5	6.0	
-	23	20	375.0	90.8	2	350.0	313.3	320.94	30.6	-2.3
	23	20	345.0	113.5	2	355.0	313.3	273.24	26.0	2.3
	24	27	350.0	72.6	12	355.0	317.8	314.56	30.0	-1.7
-	24	27	350.0	63.6	12	350.0	308.7	327.72	31.2	-2.9
28 5A	25	28	350.0	326.9	17	345.0	313.3	181.94	17.3	11.0
	25	28	345.0	227.0	17	350.0	308.7	350.38	33.4	-5.1
	26	23	350.0	77.2	24	350.0	313.3	309.56	29.5	-1 -2 -2
Empty 6B	26	23	355.0	345.0	24	350.0	345.0	14.96	4.1	
ļ	29	22	345.0	217.9						
ļ.	29	22	350.0	158.9				5 49 4		
	28	4	350.0	6.66	18	350.0	322.3	277.78	26.5	1.8
17 8B	28	4	350.0	81.7	18	350.0	313.3	305.02	29.0	-0.7
	27	16	345.0	95.3	5	345.0	313.3	281.4	26.8	1.5
	27	16	345.0	63.6	2	350.0	304.2	327.26	31.2	-2.9
-		53	350.0	59.0	19	355.0	317.8	328.18	31.3	-3.0
47 10B		53	355.0	104.4	19	350.0	313.3	287.32	27.4	6.0
-	17	7	355.0	118.0	ဇ	345.0	308.7	273.24	26.0	2.3
	17	2	345.0	104.4	တ	350.0	308.7	281.86	26.8	1.5
-	19	30	350.0	86.3	13	350.0	313.3	300.48	28.6	-0.3
39 12B	-	30	345.0	86.3	13	350.0	313.3	295.48	28.1	0.2
ľ	LS		8400.0	2760.3		7005.0	5942.9	5646.9	537.8	
Aver.			350.0	125.5		350.3	312.8	297.2	28.3	
SD			6.3	81.2		3.0	4.0	35.2	3.4	
NOTE		n cade 7 was	s exchanged	with the fee	Feeder from cage 7 was exchanged with the feeder from cage 5	5 on FD ?.			28.9	w/o #28
)		This was because the roc	rodent in 5A	was initially	tent in 5A was initially having trouble advancing the food bar.	advancing the	e food bar.		2.1	

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-	•		LS-1) mission along with the
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spacelab flown after "return	to orbit"; it was also the fir	st spacelab mission spec	rifically designated as a Life
Sciences Spacelab. The exp	eriments performed provided	l baseline data for both h	ardware and rodents used in
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